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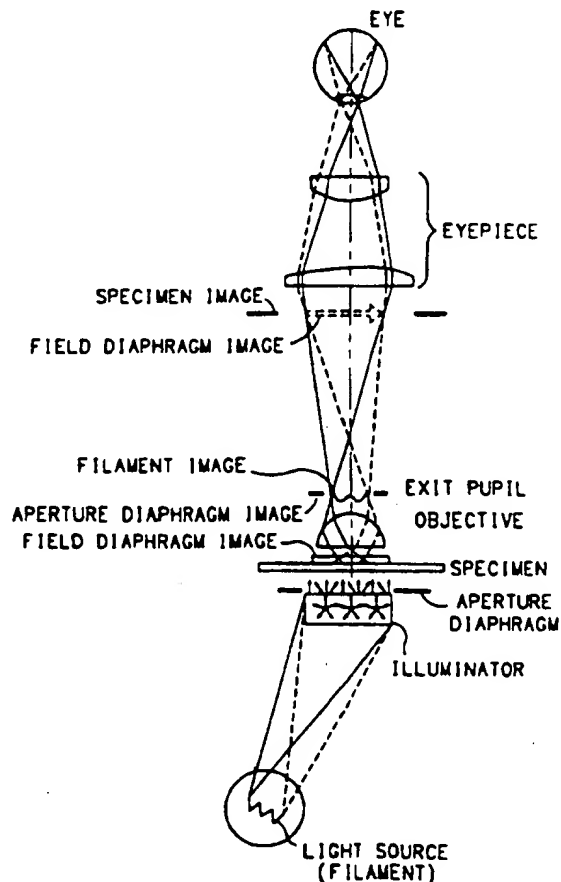
## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>6</sup> : <b>G02B 21/00, 21/06</b>	<b>A1</b>	(11) International Publication Number: <b>WO 95/30919</b> (43) International Publication Date: 16 November 1995 (16.11.95)
<p>(21) International Application Number: PCT/US95/06085</p> <p>(22) International Filing Date: 8 May 1995 (08.05.95)</p> <p>(30) Priority Data: 08/239,683 9 May 1994 (09.05.94) US</p> <p>(71) Applicant: THE REGENTS OF THE UNIVERSITY OF CALIFORNIA [US/US]; 22nd floor, 300 Lakeside Drive, Oakland, CA 94612-3550 (US).</p> <p>(72) Inventors: KRASIEVA, Tatiana; 1812 Verano Place, Irvine, CA 92715 (US). TROMBERG, Bruce; 17 Zola Court, Irvine, CA 92715 (US). DVORNIKOV, Alexander; 1812 Verano Place, Irvine, CA 92715 (US). BERNS, Michael, W.; 31132 Via Colinas, Trabuco Canyon, CA 92679 (US).</p> <p>(74) Agent: FUESS, William, C.; Suite E1, 11080 Roselle Street, San Diego, CA 92121-1223 (US).</p>		<p>(81) Designated States: JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).</p> <p><b>Published</b> <i>With international search report.</i></p>

(54) Title: ILLUMINATOR ELEMENTS FOR CONVENTIONAL LIGHT MICROSCOPES

## (57) Abstract

An illuminator element consisting of chromophores, particularly fluophores, and/or light-scattering bodies in a stable, typically a polymer plastic, matrix completely replaces an infinite set of condensers for an optical microscope, and works equally well with microscope objective lenses of any and all numerical apertures. Set of illuminator elements permit the ready production of color light(s) of any desired spectral characteristics from primary light sources that are no more sophisticated, nor any more expensive, than common electric light bulbs. Illumination fully comparable to Koehler illumination is obtained virtually effortlessly simply by placing the illuminator element near, and normally directly upon, a specimen that is typically mounted upon a microscope slide.



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## 30 ABSTRACT

## BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention generally concerns light microscopes -  
- particularly the illuminating light source and the condenser  
35 components of light microscopes -- and the techniques of light  
microscopy -- particularly the aligning of the microscope's

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illuminating light source, the focusing of the microscope's condenser onto the specimen, the setting of the aperture of the field diaphragm, and the matching the numerical aperture of the condenser to the numerical aperture of the objective.

5       The present invention particularly concerns certain improvements to light microscopes that completely eliminate the microscope's condenser, thereby obviating any necessity both for (i) aligning the illuminating light source and condenser, and (ii) matching the numerical aperture of the condenser to the  
10       numerical aperture of the objective lens. The same improvements also permit the use an inexpensive primary light source having an undistinguished, and commonly a yellowish, spectrum to produce artificial, engineered-spectrum, illuminating lights having any desired color or colors, including pseudo-white illuminating  
15       lights.

## 2.       Description of the Prior Art

### 2.1       General Background

Well-designed and quality-built modern optical microscopes often fail to produce ideal images in actual use. The primary  
20       reasons for this are: 1) improper use of the illuminating light source, i.e. non-Koehler illumination, and 2) the use of a condenser-objective pair with mismatched numerical apertures.

Microscope image quality is a complex function of several variables including: the alignment of the illuminator, the focus  
25       of the condenser onto the specimen, the aperture of the field diaphragm, and the match between the numerical aperture of the condenser and the objective. Less than perfect adjustment of any of these parameters results in degradation of image intensity, resolution and/or contrast.

30       The quality of transmitted light microscopy is inordinately dependent on the correct, skilled, use of light sources and condensers. Yet this usage is highly complex, having a long and difficult learning curve. Even after the skill is learned the adjustment of the condenser and light source of an optical  
35       microscope is a labor intensive task.

There are few skills involved in the use of an optical microscope that serve to so completely reveal the expertise and

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experience of the person using the microscope as is the adjustment of the microscope's condenser. Many persons who profess expertise with microscopes, and who often genuinely believe that they are possessed of such expertise, are inept in forming the best possible image with an optical microscope; meaning that the best image that these people are able to form, usually after much labor, is often readily visually detectable, even by an untrained eye, to be inferior to the best possible image obtainable from an optimal adjustment of the microscope by a highly skilled microscopist.

When (i) the vast numbers of microscope users, and (ii) the exceedingly modest skills level of an average user in adjusting a microscope's condenser and light source, are both considered then it is immediately obvious that many hundreds of thousands of man-hours are spent looking at, and photographing, inferior microscopic images. It might be surmised that, to such degree as these images are sub-optimal due to improper adjustment of the microscope's light source and condenser, the images are nonetheless adequate. However, quality optical microscopes are very expensive machines, ranging in price to many tens of thousands of dollars U.S. (\$10,000+ U.S.) circa 1994. Many investigators maintain that they require microscopes of the highest quality, and spend considerable amount of money on these microscopes in pursuit of better images. However, these same investigators are all too often lamentably ineffective in obtaining the best quality images with any standard microscope, regardless of cost. They are so ineffective principally because of (i) lack of the availability of a condenser having a particular numerical aperture as is optimal for a particular objective magnification, and/or (ii) lack of expertise in the delicate, complex and exacting task of adjusting the microscope's condenser and light source.

The optimal condenser has a numerical aperture greater than or equal to the numerical aperture of the objective lens. Most microscopists make, and most microscopes are ultimately used to make, microscopic observations at differing magnifications by use of greater numbers of different objective lens having different

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numerical apertures than are the corresponding numbers of matching condensers available. This is because condensers are expensive, and only rare locations have extensive sets, let alone sets of multiple condensers each of which is of the highest quality. A variety of condensers offering different levels of correction for chromatic and spherical aberration are currently available. Usually, the simplest Abbe condenser costs several hundred United States dollars (\$200+ U.S.), and the most sophisticated achromatic/aplanatic condensers having high numerical apertures cost several thousand United States dollars (\$2,000+ U.S.), circa 1994.

The number of objective lenses used during the lifetime of a microscope in use in the biological sciences varies greatly from application to application. Certain limited-use microscopes suffice to be supported by three to five (3-5) objectives and one condenser. A more comprehensive microscope application, and set, might include six to eight (6-8) brightfield (br.f.) objectives (e.g.; 2.5X, 5X, 10X, 20X, 32X, 40X, 63X, 100X), four to six (4-6) phase contrast objectives (e.g.; 10X Ph1, 20X Ph2, 32X Ph2, 40X Ph2, 63X Ph3, 100X Ph3). These objectives might normally be accompanied by four to five (4-5) condensers: a long-working distance condenser with a lower numerical aperture (e.g.; 0.33, 0.55) with brightfield of DIC and phase rings, a condenser with a higher numerical aperture (e.g.; 0.6 or 0.63), one with a highest numerical aperture (e.g.; 0.9 or 1.2 oil immersion), and one for darkfield illumination. The more extensive sets -- mandated if imaging is to be optimal under all circumstances -- are seldom found in the real world, nor can such expensive sets often be justified on the basis of cost effectiveness.

A diagram of the cone of illumination of a prior art microscope where the substage condenser and the diaphragm are properly adjusted so that the cone of illumination completely fills the aperture of the microscope objective is shown in Figure 1. Likewise, a diagram more completely showing the image-forming ray paths that are traced from ends of lamp filament of a prior art microscope is contained in Figure 2. Conjugate foci are the field diaphragm, specimen plane, intermediate image plane



(entrance pupil of eyepiece) and, with the camera in place, the film plane.

Figures 3a through 3c respectively shows prior art condensers of the abbe, aplanatic and aplanatic achromatic types. Figures 4a through 4c show the cones of light respectively transmitted by the prior art abbe, aplanatic and aplanatic achromatic condensers previously seen in Figures 3a through 3c.

Buying a more expensive microscope does virtually nothing to alleviate any (ii) lack of expertise, nor the difficult labors, that are necessary to adjust a microscope's condenser and light source to achieve optimal, Koehler, illumination. To understand (i) what Koehler illumination is, (ii) why it is difficult to achieve, and, ultimately, (iii) how the illumination to be provided by the present invention will compare to Koehler illumination, it is preliminarily necessary to discuss the optical train of existing light microscopes. This is done in the following section.

## 2.2 Detail Background

A detailed discussion, culminating in a detailed explanation of the adjustment, and the considerable sophistication and difficulties of the adjustment, of an optical microscope for Koehler illumination may be found in the book *Microscopes: Basics and Beyond*, Volume 1, by M. Abramowitz, available from Olympus Optical Corporation. The present discussion is abridged from that book in order that (i) Koehler illumination may be clearly understood and defined, (ii) existing techniques for the use of a microscope condenser may be definitively set forth, and (iii) the optical paths of the present invention, as later explained, may be rigorously compared with the optical paths of previous optical microscopes having a condenser.

### 2.2.1 The Essential Optical Train of the Light Microscope

The compound light microscope provides a two-dimensional magnified image of the specimen that permits resolving and measuring fine details of the specimen structure. The specimen can be positioned (oriented) and focused precisely, and the contrast and brightness of the image can be adjusted to bring out desired features of the specimen structure.

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The optical train of a microscope usually consists of: (1) the illuminator, including the light source and collector; (2) the condenser; (3) the specimen, including the slide and coverslip; (4) the objective lens; (5) the ocular, or eyepiece; and (6) the camera, or observer's eye.

In addition, a conditioning device is often inserted between the illuminator and the condenser and a complementary filtering devices together modify the image contrast as functions of spatial frequency, phase, polarization, wavelength, etc.

Whether or not specific devices are inserted to condition the entrant waves and to filter the image-forming waves, some conditioning and filtering naturally takes place in any microscope.

Some of the components in the optical train of the light microscope act as imaging elements, while most also have filtering or transforming functions. The components that form images in the microscope optical train are: the collector and the illuminator, the condenser lens, the objective lens, the ocular and the refractive elements of the eye, or the lens on the camera.

As their names imply, some of these components are not commonly thought of as imaging components. Nevertheless their properties are important in determining the final quality of the microscope image.

## 2.2.2 Principles of Koehler Illumination

The condenser in the light microscope was initially developed (as the name implies) for collecting ample light to illuminate the specimen. While many individuals still view this as the main function of the condenser, in fact the "illuminating system" plays a far more important role in microscopy than is commonly recognized. The role of the illuminating system is indeed so important that one can quickly spot the degree of sophistication of the user of a microscope by the way the condenser and illuminator are adjusted.

One can achieve a superb image by properly aligning the axes, and adjusting the foci and diaphragm openings in the light source and the condenser. On the other hand, by poorly adjusting

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the optical train that illuminates the specimen, one can use a \$2000 objective lens and produce an image that is worse than that produced by a \$200 objective lens used skillfully. Of course, a more expensive, better-corrected objective lens can provide an image that is vastly better than a lower-priced lens if the illuminating system is adjusted well. One can equip modern research microscopes with condensers that are well corrected for aberrations (e.g., aplanatic achromats). In addition to selecting a superior-quality condenser, it is important to use the condenser under the specified optical conditions; either dry or oil-immersed; with proper distance between the condenser and the field diaphragm; and fully adjusted for Koehler illumination, with the aperture uniformly and fully illuminated.

In adjusting the illuminating system, or the light waves that illuminate the specimen in a microscope, Koehler illumination (Koehler, 1893; Dempster, 1944) provides several advantages.

First, the field is homogeneously bright. Second, the working numerical aperture of the condenser and the size of the illuminated field can be regulated independently. Third, the specimen can be illuminated by a converging set of plane wave fronts, each arising from separate points of the light source imaged in the condenser aperture. Fourth, this gives rise to the maximum lateral resolution and very fine optical sectioning, which yields maximum axial resolution. Fifth, the front focal plane of the condenser becomes conjugate with the rear focal plane of the objective lens, a condition needed for optimal contrast enhancement of the finer specimen details. Sixth, flare, arising from the microscope optics and their barrels, is reduced without any vignetting.

Many of these benefits, insofar as they are pertinent (the second and fifth elements will not prove to be so pertinent), will also be seen to be realized by the illuminator elements of the present invention -- even though such illuminator elements are vastly different than the condensers that they replace.

For Koehler illumination, the various lenses of the microscope are arranged as shown in Figures 5 and 6 and are

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focused as follows.

First, the collector lens ( $L_s$ ) focuses an image of the light source onto the condenser iris diaphragm ( $D_c$ ). Second, the condenser lens ( $L_c$ ) focuses an image of the field diaphragm ( $D_s$ ) in the plane of the specimen. Third, the objective lens, the ocular, and the refractive elements of the eye (or camera) together focus an image of the specimen onto the retina of the observer's eye (or the camera image plane).

These adjustments give rise to the two sets of optical paths and the image planes represented in Figures 5 and 6. Together, the two sets of optical paths and the image planes characterize Koehler illumination.

Each set of optical paths and image planes will now be examined in turn, starting with Figure 5.

As shown in Figure 5, the collector lens of the illuminator produces an enlarged inverted image ( $S_2$ ) of the light source ( $S_1$ ) onto the condenser iris diaphragm.

Then the condenser and the objective lens together form an image ( $S_3$ ) of  $S_2$  onto the rear focal plane of the objective lens in the following manner.

The condenser of the microscope is designed so that its iris diaphragm is located at the front focal plane ( $F^1$ ) of the condenser lens. See Figure 5. Therefore, light emanating from a point in the plane of the condenser iris emerges from the condenser as parallel rays, or as a plane wave.

As shown in Figure 5, this plane wave traverses the specimen space and enters the objective lens. The objective lens converts the plane wave to a spherical wave. The spherical wave converges to the rear focal plane of the objective lens ( $S_3$ ).

Thus, each point at the rear focal plane of the objective lens is conjugate with a corresponding point in the plane of the condenser iris diaphragm, and the condenser and objective lenses together form an inverted real image --  $S_3$  -- of  $S_2$  at the rear focal plane of the objective lens. See Figure 5.

The rear focal plane of the objective lens or, to a close approximation, its back aperture ( $D_{ob}$ ) is in turn focused by the ocular (see Figure 5), which forms a small inverted image ( $S_4$ ) at

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$D_{ob}$  ( $\approx S_3$ ) at the eye point.

The eye point is located just beyond the rear focus ( $F_{oc}$ ) of the ocular; it is where the pupil of the eye is placed.

In this manner, a series of planes containing  $S_1$ ,  $S_2$ , and  $S_3$  are successively focused and relayed to the eye point by the lenses  $L_s$ ,  $L_c$ , plus  $L_{ob}$ , and  $L_{oc}$ . At the eye point, these images are all superimposed in the same plane and appear as the Ramsden disk.

For each lens (or lens group), the image and its source can be interchanged and each image and its source lie in conjugate planes. This means that a source of light, an image, or an object placed in any one of the four planes ( $S_1$ - $S_4$ ) would also be focused to the other three planes.

Therefore, in Koehler illumination, the following four structures lie in conjugate planes: the light source, the condenser iris diaphragm, the rear focal plane of the objective lens, and the eye point. These four lie in the **aperture planes** of the light microscope.

Simultaneously, another set of conditions represented in Figure 6 is fulfilled in Koehler illumination.

As shown in Figure 6, the condenser lens ( $L_c$ ) produces a small inverted image of the field diaphragm onto the plane of the specimen. The field diaphragm is located at  $I_1$ , and the specimen at  $I_2$ .

The specimen is then focused by the objective lens ( $L_{ob}$ , see Figure 6), which produces a magnified inverted image ( $I_3$ ) of the specimen.  $I_3$  appears in the intermediate image plane containing the field stop ( $D_{oc}$ ) in the ocular.

Next, as shown in Figure 6, the lenses of the ocular ( $L_{oc}$ ) and the refractive elements of the eye ( $L_c$ ) together form an image ( $I_4$ ) on the retina.  $I_4$  is inverted relative to  $I_3$ .

Therefore, in Koehler illumination, the following four structures also lie in conjugate planes: the field diaphragm, the specimen, the ocular field stop, and the retina. These four conjugate planes are the field planes.

In Koehler illumination, then, we have two sets of conjugate planes: the aperture planes  $S_1$ - $S_4$ , and the field planes  $I_1$ - $I_4$ .

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The two sets of conjugate planes are related to each other in an important fashion as follows.

As shown in Figure 5, the spherical wave fronts converge, and the rays focus, onto the aperture planes ( $S_1$ - $S_4$ ). As shown in Figure 6 the wave front converge and focus onto the field planes ( $I_1$ - $I_4$ ).

On the other hand, the wave fronts shown in Figure 5 are planar, or nearly so, as they traverse the field planes. Likewise, the wave fronts shown in Figure 6 are also planar, or nearly so, as they traverse the aperture planes.

Therefore, spherical waves in one set of conjugate planes become (nearly) plane waves in the other set of conjugate planes, and vice versa. Rays that are focused in one set of conjugate planes are (nearly) parallel rays in the other.

The two sets of conjugate planes are thus reciprocally related to each other.

This reciprocal relationship explains how the various diaphragms and stops in a microscope affect the cone angle of illumination and the size, brightness, and uniformity of the microscope field (see the next section 2.2.3). Whatever changes can be made to the beam of light in the field planes (i.e., by manipulating apertures or angles of the light path or by inserting conditioners) also can be made in the aperture planes by changing the angle or opening for the beam. The relationship is reciprocal.

More fundamentally, the reciprocal relationship between the two sets of conjugate planes explains how the light waves illuminating the specimen and diffracted by the specimen relate to each other. These relationships in turn explain the function and adjustments of the devices that condition the entrant wave and filter the specimen image.

All of these parameters together determine (or affect) the resolution, contrast, and fidelity of the microscope image.

### 2.2.3 Adjusting the Microscope for Koehler Illumination

The goal of Koehler illumination is to align all of the optical components on axis, to focus the lenses correctly, and to adjust the diaphragms appropriately. The prior art

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illuminator will be considered first.

#### 2.2.3.1 Alignment and Focus of a Prior Art Illuminator

Misalignment of a prior art illuminator can give rise to an unevenly illuminated field of view or an unevenly lit aperture. An unevenly illuminated field can be particularly troublesome in video microscopy where image contrast is enhanced electronically; unevenness of the field is accentuated together with the contrast of the specimen.

Although an unevenly illuminated aperture will not necessarily affect field uniformity, it can critically affect the quality of the image. Therefore, the light source and the whole illuminator need to be properly aligned, as well as correctly focused, for Koehler illumination.

For various reasons, the collector lenses in a prior art illuminator are rarely corrected very well. This imperfection may be used to advantage to center the light source with respect to the collector lenses.

#### 2.2.4 Darkfield Microscopy, Rheinberg Illumination and Optical Staining

The present invention will be seen to provide very strong support, albeit in an entirely new manner, for darkfield microscopy, Rheinberg illumination and optical staining. Darkfield microscopy, Rheinberg illumination and optical staining are discussed by the aforementioned Mortimer Abramowitz in another of his books: Contrast Methods in Microscopy - Transmitted Light, Volume 2, from the Olympus Corporation, Japan. The following discussion is drawn from the "Contrast Methods" section of that book.

Darkfield microscopy is based on the same principle by which the stars, invisible to the eye in the bright daytime sky, become visible at night because of the stark contrast between the faint light and the black sky. This principle is applied in darkfield (also called darkground) microscopy as a simple and popular method of making unstained objects clearly visible. Such objects are often barely seen in conventional brightfield microscopy.

Darkfield illumination requires the blocking out of the central light which ordinarily passes through or around (the

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surround) the specimen -- and allowing only oblique rays to "strike" the specimen mounted on the microscope slide. See Figures 7a and 7b.

5 If no specimen is placed on the slide, especially an unstained, non-light absorbing specimen, the oblique rays cross the specimen and are diffracted or refracted so that these faint rays are able to enter the objective; the specimen can then be seen bright on an otherwise black background. As in the example of starlight described above, the visibility is greatly enhanced  
10 by the contrast between the brightly shining specimen and the dark surround.

What has happened in darkfield illumination is that all the ordinarily un-deviated rays of the zero'th order have been blocked; the oblique rays, now diffracted by the specimen and  
15 yielding first, second, and higher diffracted orders at the back focal plane of the objective, proceed onto the image plane where they interfere with one another to produce an image of the specimen. See Figure 7c.

20 Darkfield objects are often quite spectacular to see (e.g. a drop of fresh blood in darkfield); objects of very low contrast in brightfield shine brilliantly in darkfield. Such illumination is best for revealing outlines, edges, and boundaries; darkfield illumination is less useful in revealing internal details.

25 A striking variation of low to medium power darkfield is known as Rheinberg illumination, first demonstrated by the British microscopist Julius Rheinberg nearly a hundred years ago. This method produces beautiful colored images of unstained objects. (cover photograph) in this form of illumination, the central opaque stop is replaced with a transparent, colored,  
30 circular stop inserted into a transparent ring of contrasting color. These stops are placed under the bottom lens of the condenser; the specimen is rendered in the color of the ring; the background is the color of the central spot. For example, a green central stop inside a red ring will show "red protozoa"  
35 swimming in a "green sea." (See Needham's "Practical Use of the Microscope" pages 281-285 for further information.)

Darkfield illumination and Rheinberg illumination are both



examples of how images are affected by manipulating light at the substage condenser. When an image is made to appear in color without use of chemical stains, the technique is described as "optical staining." The present invention will be seen to provide superior support for optical staining.

#### 2.2.5 Film, Color Temperature, Color Staining, and Color Filters, and Prior Art Color Image Rendition

The present invention will be seen to offer remarkable control over color during micrography, photomicrography and color photomicrography.

The color of a light source or its difference when color photomicrography is performed is indicated by its "color temperature". This term arises from the concept that when a black iron bar is heated, or carbon (charcoal) is burnt, it progressively becomes red, yellow, white and blue with successive increases in temperature. In stead of an iron bar or charcoal, the measurement of "color temperature" uses a fictitious, ideal, substance called a "full radiator", and the radiation energy of this substance is used as a standard. The "color temperature" of a real-world light source is equal to the absolute temperature, in degrees Kelvin, of a radiating full radiator that would produce the same color light.

For example, the color temperature of the earth's blue sky is from 8000 K° to 10000 K°; the sun at noon from 5500 K° to 6500 K°; a twelve volt, fifty watt (12 V, 50 W) halogen lamp 3400 K° (at 12 V); and a six volt, thirty watt (6 V, 30 W) tungsten lamp 2850 K° (at 7.5 V).

There are two major types of color film for all purposes including color photomicrography. Daylight-type color film is balance for sunlight. Tungsten-type color film is balanced for artificial light. The selection of the film used is dependent (prior to the present invention) on the light source used. This selection is important. Photographs will appear excessively red if daylight film is used under incandescent light conditions. Similarly, when exposing a tungsten-type film with daylight a bluish photograph is obtained. The proper color temperature to expose daylight-type color film is typically 5500 K° to 6000 K°;

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to expose tungsten-type color film is typically 3200 K° or 3400 K°.

In order to correct the color temperature of the image to the film, color filters are used.

5       The present invention will be seen to offer great ease, and certainty, in matching the illuminating light to any color film of choice during color photomicroscopy.

10       Color contrast filters are also used to control the contrast within black-and-white photographs. The filter that enhances the contrast within a black-and-white photograph of a red/yellow specimen is green. The filter that enhances the contrast within a black-and-white photograph of a yellow/orange specimen is blue. The filter that enhances the contrast within a black-and-white photograph of a blue specimen is orange.

15       Color contrast filters were the early, and are the presently-used, method of increasing contrast of stained specimens. The color contrast filters are placed in the light path. For example, if a specimen is stained with a red stain, a green filter will darken the red areas thus increasing the contrast. On the other hand, a green filter would lighten any green stained area.

20       Each of light-balancing filters, and color-compensating filters, will be seen to be substantially obviated, and supplanted, by the microscopy illumination light sources of the present invention.

### 25       2.3       Summary of the Limitations of Prior Art Microscope Condensers and Light Sources

30       One condenser, howsoever expensive, seldom suits all applications in which a microscope is used. The numerical aperture of the condenser should optimally be matched to the numerical aperture of each objective lens as may, from time to time, be used in the microscope. This normally requires that not just one, but several, expensive condensers are required in support of each microscope. The several condensers are collectively so expensive as to typically aggregate a significant percentage of the total cost of the microscope on which and with which they are used. Because only one condenser is in use at any

one time on any one microscope the collective condensers represent, in aggregate, a costly resource that has a low average usage.

Moreover, the adjustment of even the correct condenser and  
5 its accompanying light source to realized Koehler illumination  
is difficult and time-consuming.

Accordingly, it would be exceedingly useful if some improvement to microscope imaging technology was to be able to alleviate the (i) expense and (ii) burden of selecting and adjusting a microscope's light source and condenser for optimal illumination, possibly by completely eliminating the condensers and/or the light source. With such an imaging technology improvement (i) the cost of the microscope could be substantially reduced by up to the cost of its condensers and/or light source, and (ii) the considerable recurring labor costs of condenser and light source alignment for Koehler illumination could be avoided.

## SUMMARY OF THE INVENTION

The present invention contemplates illuminator elements for use with optical microscopes. Each and any individual illuminator element completely replaces an infinite set of condensers for a microscope, and works equally well with microscope objective lenses of any and all numerical apertures.

Furthermore, in certain (preferred) embodiments of the illuminator elements that are used in combination with a primary source of light that is external to the illuminator element itself, this external light source replaces the normal specimen illumination light source of the microscope.

Many illuminator elements may be related as a set where each different one of a number of related illuminator elements produces a precisely "spectrally-engineered" colored light(s) of a predetermined spectrum or spectra, including a spectra of a pseudo-white light. (A number of illuminator elements accordingly form a "set" for purposes totally unrelated a "set" of prior art condensers having differing numerical apertures.)

35     Sets of such illuminator elements permit the ready production  
- ... of light(s) of any desired spectral characteristics from primary

light sources that are no more sophisticated, nor any more expensive, than ordinary household alternating current (a.c.), or flashlight direct current (d.c.), incandescent light bulbs.

1. Shape, Size and Appearance of the Illuminator Elements of the Present Invention

In their most general form, illuminator elements in accordance with the present invention are used with optical microscopes that detect light radiation in the range from the near infrared (NIR) to the ultraviolet (UV). An illuminator element for these microscopes is typically a small and compact, unitary, and unchanging piece of material that is devoid of moving parts.

Illuminator elements may be, and are, formed in myriad shapes and volumes. It is necessary only that an illuminator element should be sized and shaped so as to be suitably positioned proximately to, and normally in contact or in partial contact with, a specimen that is located upon the specimen stage of an optical microscope. According to this requirement, illuminator elements commonly have a volume from a fraction to a few cubic centimeters ( $\text{cm}^3$ ). They typically (but not necessarily) have the regular geometric shapes of polyhedrons (such as cubes, or parallelepipeds, or prisms), oblate spheroids, or, most commonly, cylinders or disks. So shaped and sized, illuminator elements commonly range in thickness from a thick film having a thickness of some fractions of a millimeter to three-dimensional bodies having a thickness of several tens of millimeters, and are more commonly about a centimeter in thickness. Each illuminator element typically has and presents an area of some fraction of a square centimeter to some few square centimeters ( $\text{cm}^2$ ) upon each of its major face(s).

The illuminator elements, while undistinguished in either size or shape, may, in some embodiments, show fairly dramatic optical properties -- even under normal ambient lighting. Some embodiments contain fluorescent compounds (as will be explained), and glow brightly like jewels. Other embodiments contain light-scattering bodies (as will also be explained), and commonly appear translucent in the manner of, for example, a milk white

opal. Many, even most, embodiments contain both fluorescent compounds and light-scattering bodies, and show a diffuse, gentle, and typically colored glow.

2. First Function of the Illuminator Elements of the Present Invention -- Substantially Isotropic Illumination

All the variously shaped and sized illuminator elements of the present invention function as a substantially evenly-spatially-distributed, substantially randomly-spatially-distributed array of a great multiplicity of substantially-point-sized light sources. Each of the light sources produces light that is (i) substantially uncorrelated in time (phase) with the light of other point light sources, and (ii) without distinction as to direction, meaning isotropic. The light that is emitted from such an illuminator element is called "spatially isotropic light". "Spatially isotropic light" is defined as light that is emitted in all directions equally, and without distinction or preference.

A common example of a source of spatially isotropic light is the phosphor surface of a common fluorescent light tube. Spatially isotropic light, and light illumination, is sometimes called "distributed Lambertian" illumination after John Lambert, 1619-1683, English parliamentarian and investigator in optics.

An illuminator element in accordance with the present invention is positioned (i) closely proximate to a specimen under observation by a microscope (ii) on an opposite side of the specimen to that side whereat exists the microscope's objective lens. For all practical purposes the illuminator element bathes the specimen in a uniformly bright, directionally isotropic, light from nearly a full two pi ( $2\pi$ ) steradians of solid angle. The specimen is thus "substantially isotopically illuminated", and, for the purpose of viewing along an optical axis through a microscope, the specimen is so substantially isotopically illuminated (to visible limits of human perception) by a homogeneously bright high-numerical-aperture light source.

3. Second Function of the Illuminator Elements of the Present Invention -- Predetermined Color(s) of Illumination

Moreover, in certain preferred embodiments of the invention,

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each individual illuminator element produces its isotropic illumination at virtually any desired, predetermined, spectral frequency or frequencies -- i.e., at any desired color or colors.

The color or colors of a color illuminator element are, in an embodiment of a first type called a "fluorescent color illuminator element", derived from the emission of fluorescent light.

The particular color or colors at which an individual color illuminator elements of the first type serve to emit light is predetermined by choice of the particular fluorescent chemicals that are incorporated in the construction of each such illuminator element. Once predetermined, the chemically-determined fluorescent light emission is typically constant and invariant throughout an indefinitely long lifetime. The fluorescent chemicals within the color illuminator element are energized to emit fluorescent light at one or more predetermined frequencies by an absorption of energy, typically a radiant energy and more typically light energy.

This energy is received from a primary external (light) radiation source. This external radiation (light) source need have no predetermined, nor any constant, spectrum. Neither, within broad ranges, need it have a particularly high, nor a uniform, intensity of radiation (light) emission. It is sufficient only that primary radiation (light) source should supply radiation within a broad spectral bandwidth to the fluorescent, color, illuminator element. The external primary radiation (light) source may typically be a common incandescent light bulb -- such as is used in a household lamp or a flashlight -- having any of (i) a brightness, (ii) a yellowish spectral output, and/or (iii) spatial properties that were previously manifestly unsuitable for use in microscopy.

The color or colors of an illuminator element are, in an embodiment of a second type -- called a color illuminator element with dye(s) and scattering bodies -- derived from (i) selectively absorbed (i.e., conversely, selectively transmitted), and from (ii) scattered, light.

The color or colors of the second-type illuminator element

are a consequence of its selective light absorption (selective light transmission). The color or colors at which an individual second-type illuminator element absorbs light (transmits light) is predetermined by the choice of chemical dyes that are incorporated in its construction. The chemically-determined light absorption (light transmission) is typically constant and invariant throughout an indefinitely long lifetime.

Dye-containing illuminator elements are illuminated, absorbing light at predetermined frequency(ies), by broad-spectrum radiant light energy developed in a primary external light source. This external light need have no predetermined, nor any constant, spectrum. It must, however, include at least the frequency(ies) that are transmitted by the chemical dye(s) that is (are) within the illuminator element, and it normally also includes frequencies that are absorbed by this dye (these dyes). Neither, within broad ranges, need the primary light source have a particularly high, nor uniform, intensity of light emission. It is sufficient only that primary light source should supply radiation containing the frequency(ies) that are transmitted by the chemical dye(s) in the illuminator element. The external primary light source is again typically a common incandescent light bulb having (i) the brightness, and (ii) the yellowish spectral output, and (iii) the spatial properties that render it manifestly unsuitable for microscopy.

The second-type illuminator element also incorporates light scattering bodies. These light-scattering bodies are typically minute agglomerations, suitably called microspheres. The light-scattering bodies (i) have a diameter on the order of the wavelength(s) of the primary external light radiation. They are (ii) made of a material having a high index of refraction. They are (iii) substantially evenly (iv) substantially randomly distributed (v) at a density that makes the scattering length of the light that they serve to scatter to be much, much smaller than the macroscopic dimensions of the illuminator element, and on the order of micrometers. An interaction between the primary external radiation, the absorbing dyes, and the light-scattering bodies causes the second-type illuminator element to produce an

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isotropic light the chromaticity of which is a function of the transmission characteristics of (i) the absorbing dye(s) and (ii) the spectral features of the primary external radiation source.

5 A third type of illuminator element contains only these light-scattering bodies (and no chromophores in the form of either fluorescent chemicals or dyes) and is so called. This type of illuminator element is a "color" illuminator element only in that it serves to render isotropic a primary external light radiation that is colored, and to assume the color(s) of this  
10 external light source, and not because it serves to impart color in of itself.

The light-scattering bodies within the third-type illuminator element are again typically of dimensions, or a dimension, on the order of the illuminating wavelength(s). They  
15 are again made of material having a high refractive index. The light-scattering bodies are dispersed in a substantially random, substantially even, spatial distribution within the body of the illuminator element. They are of such a density as causes myriad light reflections, with the scattering length being on the order  
20 of micrometers. The light-scattering bodies serve to spatially redistribute and randomize the external light source by multiple scattering occurring along a great number of essentially random paths.

Still another, fourth and major, type of illuminator element  
25 contains both fluorescent chemicals and light-scattering bodies, and is so called. The fluorescent chemicals are normally capable of reabsorbing, albeit with less than 100% efficiency, their own fluorescent light as well such light as is received from the external, primary, radiation source. Multiple absorptions and  
30 emissions produce a particularly pure and diffuse, isotropic, colored light output.

All the illuminator elements such as produce a particular color(s) of light may usefully be associated with certain, spectrally related, color dyes that are used to stain the  
35 specimen. The dye(s) used to stain the specimen are normally chosen so as to have a very pure, spectrally sharp, color (of reflection). Meanwhile, the spectrum of the illuminator element



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is correspondingly sharp, and overlaps the reflection spectrum of the dye(s). In microscopically observing a color-dyed specimen with this narrow-spectrum light the selectively color-dyed portions of the specimen will be optically isolated from the general (light) background at a tremendous "signal-to-noise", and will stand out exceedingly clearly.

A color illuminator element is not limited to emitting, to absorbing (transmitting), or to scattering at only one frequency, but can emit, absorb or scatter at several frequencies simultaneously -- even while excited from but a single external primary radiation source. For example, one preferred emission-type illuminator element in accordance with the present invention produces narrow spectral bandwidth emission peaks at each of the red, blue and green primary colors: thus a pseudo-white light. In observing an unstained or neutrally-colored specimen, some microscopists prefer this pseudo-white light to broad-spectrum common incandescent "white" light, or daylight. However, if the specimen is, per chance, selectively stained red, blue and green (each in an associated receptor structure within the specimen), then observation under the pseudo-white light will be notably different than with regular, spread-spectrum, white light. Namely, the stained regions will appear very sharply contrasted and prominent (as would be expected).

#### 4. Construction of Certain Preferred Embodiments of the Illuminator Elements

Each illuminator element of the present invention functions to emit or to scatter light isotopically and in all directions equally because (i) a multiplicity of (essentially) isotopically absorbing/emitting, absorbing/transmitting, and/or simply reflecting (essentially) molecular-size, light sources are (essentially) evenly spatially distributed within (ii) a stable matrix suitably sized and shaped so as to be positioned closely proximate to a specimen under observation by a microscope.

Typically the light sources are (i) fluorescent molecules, (ii) dye molecules and/or (iii) light-scattering particles in a size range on the order of the wavelength(s) of the light that they serve to emit, to absorb (transmit) and/or to scatter, and

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are more typically in a size range from .5 to 1.5 microns. The density of these light sources is sufficiently high so that the path of light between (i) emission, absorption, and re-emission, and/or between (ii) emission and scattering, and/or between (iii) successive scatterings (i.e., the scattering length) is very short, and on the order of micrometers.

The distribution of these multiple light sources within the matrix is typically random and homogeneous, although it is not necessary either. (When the distribution is not random, and/or when the density is not uniform, the directional isotropy of the light is not necessarily destroyed nor even degraded, but the intensity (amplitude) of the produced light will typically incur a gradient across the illuminator element.)

In its detail structure, an illuminator element of the present invention is based on a matrix that is stable for at least the period of microscopic observation, and that is more commonly solid and stable over an indefinitely long period. The matrix is typically made from translucent or transparent (at the light frequency or frequencies of interest) polymer plastic or glass. The matrix is more typically made from polymer plastic, and particularly from polymethylmethacrylate and poly-2-hydroxy ethylmethacrylate in proportion 3:1 by volume.

Of much greater interest, and much greater sophistication, than is the matrix -- which is but the support mechanism for the producers of isotropic light -- are the various substances and mechanisms by which the isotropic light illumination is produced, especially at a certain predetermined frequency or frequencies.

There are various ways to categorize the principle embodiments of the illuminator elements in accordance with the present invention. Moreover, various individual illuminator elements may be classified in multiple categories -- howsoever the categories are variously defined. The following sub-sections 4.1-4.4 categorize the illuminator elements by (i) the chemicals contained therein, and by (ii) the density of such chemicals. In this categorization, a first major type of illuminator element produces spatially isotropic light illumination by action of great numbers of (i) chromophores, defined as a chemical group

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that gives rise to color in a molecule. In this categorization, another major type of illuminator element produces isotropic light illumination by action of great numbers of (ii) substantially-non-absorbing highly-reflective light-scattering  
5 bodies that collectively isotopically scatter light received from a primary external source thereof onto the specimen at low loss. In this categorization, still another type of illuminator element produces isotropic light illumination by (iii) a combination of chromophores and light-scattering bodies.

10 Both the chromophores and/or the light-scattering bodies collectively serve to randomize an excitation light -- which is distinctly directional and non-isotropic -- that is received from an external source. This external light is transformed into an essentially uniform distribution of plane waves arising from  
15 separate points that are distributed (i) essentially uniformly, (ii) essentially continuously, and (iii) essentially randomly in space. Such a distribution of plane waves constitutes one definition of "isotropic illumination", or of an "isotropic light source".

20 An illuminator element in accordance with the present invention does not normally have an equal index of refraction to any of the material of (i) a microscope slide, (ii) a specimen, (iii) a cover slip, or (iv) any oil or water in which the specimen may be contained. Accordingly, isotropic light  
25 illumination exiting the surfaces of the illuminator element is diffracted before impinging upon the specimen. Light rays arise within the illuminator element at greater than a critical angle to its surface will be internally reflected, and will not impinge upon the specimen at all. Accordingly, the specimen does not  
30 receive such precisely isotropic illumination as would only be produced by an illuminator element of infinite size, is best spoken of as being "substantially" isotopically illuminated over a broad angle. The definition of what constitutes "substantiality" is, however, quite simple: the specimen-  
35 illuminating light is so "substantially" isotropic that no shadows are visible through the microscope.

Moreover, the chromophores (within illuminator elements

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having such chromophores) serve to select and/or to transform in frequency the light received from the primary external source thereof.

5 Notably, the primary external light source may be derived from almost any crude source of light that is shined upon the illuminator element from almost any angle (save along the optical axis of the microscope). The primary external light source most particularly need not be the normal specimen illumination light source of the microscope (although this generally expensive  
10 source will suffice admirably). The primary external light source may be, for example, (i) a standard 110 v.a.c. incandescent light shielded so as to shine upon the illuminator element, or even (ii) a common pocket pen light, or flashlight.

15 With these general principles in mind, the following categories of illuminator elements in accordance with the present invention may be considered.

4.1 Illuminator Elements With Chromophores, Particularly Luminescent Chromophores, and More Particularly Fluorescent Fluorophores

20 If chromophores are used in the illuminator element, then they may either be of (i) a chemical group having the aspect of a color light source (i.e., a light that is describable in terms of hue, brightness and saturation, or (ii) a chemical group having the aspect of a colored object (i.e., an object that is  
25 describable in terms of hue, lightness and saturation).

If the chromophores used are of the character of a chemical light source then they are, by definition, luminescent chromophores. The luminescent chromophores may be any of (i) radioluminescent, (ii) chemiluminescent, or, preferably, (iii)  
30 fluorescent. Fluorescent luminescent chromophores are equivalently called either fluorochromes or fluorophores.

In those embodiments of the present invention using fluorescent fluorophores (which are also called fluorescent chromophores, and fluorochromes), the fluorophores are excited  
35 with radiation from an external source thereof so as to emit a non-directional isotropic fluorescent light by which the specimen may be substantially isotopically illuminated. The emitted

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fluorescent light has a predetermined optical spectrum. One common emission spectrum preferably has but one, narrow, emission peak substantially at but one single frequency (i.e., one color). For example, the fluorophore rhodamine 125 produces the color green with a peak emission at an approximate wavelength of 524 nanometers.

Alternatively, a fluorophores of several different types may be used to emit, normally in response to the same excitation radiation, fluorescent light each at an associated optical spectrum. The composite emitted light spectra formed by the combination of the several optical emission spectrum of each different type fluorophore constitutes an artificially engineered -- commonly a pseudo-white -- light. The preferred pseudo-white light has only a few, typically three, spaced-apart emission peaks. It has been found that some microscopists prefer such a pseudo-white light for observations, as opposed to observing under the broadly smeared spectrum of normal sunlight or of artificial light. One particularly effective pseudo-white light is comprised of equal intensities of green, blue and red light. The green light is made from rhodamine (both types 6G and 110) fluorophores emitting light within a sharp peak (on the plot of emission versus frequency) centered about 550 nanometers; the blue light from coumarin fluorophores emitting light within a sharp peak centered about 420 nanometers; and the red light from sulforhodamine fluorophores emitting light within a sharp peak centered about 610 nanometers. Clearly any desired colored light or lights as suit a particular microscopic observation can be made from particular fluorophores, of which there are many well-known and well-documented types.

The present invention also contemplates entire families of related illuminator elements, each of which elements emits a particular color or colors of light (including pseudo-white lights).

The chromophores that are of the character of a chemical light source, and particularly the fluorophores, may be, and preferably are, combined in the matrix with substantially highly-reflective light-scattering bodies. The light-scattering bodies

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are commonly made from a material having an index of refraction that differs greatly (i.e.,  $> X2$ ) from the index of refraction of the stable matrix in which the light-scattering bodies are contained, and are more commonly made from titanium dioxide. In use in combination with fluorophores, the light-scattering bodies collectively serve to aid in isotopically scattering the luminescent, fluorescent, light onto the specimen.

These same highly-reflective light-scattering bodies will soon be seen to have yet another, related, use in another, major, embodiment of the present invention yet to be discussed. Although the light-scattering bodies serve to scatter light, and to increase isotropy, in all applications, when the use of these bodies in various further embodiments of the invention is later considered and compared, it will be useful to then think about precisely just what light(s) the bodies are primarily serving to scatter, and not only that the bodies serve to scatter light.

#### 4.2 Illuminator Elements With Chromophores, Particularly Pigments, Accompanied by Scattering Bodies

Chromophores also include chemical groups having the aspect of a colored object, defined as an object describable in terms of hue, lightness and saturation. Those embodiments of illuminator elements in accordance with the present invention employing chromophores of this type -- namely one or more colored chemicals, or pigments, that absorb some, and that reflect some, of light received from an external source of light -- also incorporate scattering bodies.

Each colored chemical, or pigment, assumes, by the well known principles of color, the color(s) of only that frequency (those frequencies) of light that it serves to reflect. Just as with the tailoring of the emission spectrum or spectra of the luminescent chromophores (predominantly fluorescent fluorophores), the absorption spectrum, or spectra, of the colored chemical(s) or pigment(s) may also be tailored to a microscopy task at hand. A colored chemical may be chosen so as to absorb light of a predetermined optical spectrum, the transmitted light spectrum having a peak substantially at but a single color. Alternatively, several colored chemicals may

chosen so as to absorb light at an associated number of different optical spectrums, with a composite transmitted light spectra being formed by a combination of all the plurality of spectrums. This composite spectra is commonly a pseudo-white light. Note  
5 that this spectrally superior light may be obtained, just as with the emitted fluorescent light, by illumination of the light source with a common broad-spectrum light source that is not itself of good spectral quality such as, for example, an incandescent light source. (Such intensity losses, and heating,  
10 as are experienced from the non-reflection of certain light frequencies are normally inconsequential.)

It will also be recognized by a practitioner of photochemistry and photochromism that the reflectivity versus frequency, or the color and the color intensity, of pigments can  
15 intentionally be controlled so as (i) to produce "soft" light, (ii) to take away, sharpen, or attenuate, some portion or portions of the spectrum (or spectra) of colored light(s) otherwise being produced by fluorophores, and/or (iii) to better accommodate (by selective attenuation) the use of an external  
20 primary light source that is not well-suited to the human eye, and that is typically excessively yellow.

The necessary scattering bodies of the illuminator element serve to scatter, diffuse and randomize the light transmitted by the colored chemicals, or pigments. Ultimately, and after  
25 multiple reflections, the transmitted light is randomized, and isotropic in nature when and where exiting the surfaces of the illuminator element.

#### 4.3 Illuminator Elements With Light-Scattering Bodies

Finally -- and as a major alternative embodiment of the  
30 invention to the use of chromophores (of either the color emissive or color reflective types) -- an illuminator element of the present invention may consist of a great number of light-scattering bodies contained within a stable matrix.

In this embodiment the light-scattering bodies collectively  
35 serve to isotopically scatter light received from the external source thereof onto the specimen at low loss. By this light-scattering action a specimen may be indirectly, isotopically,

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illuminated by an external source of light that may itself be highly anisotropic. Such anisotropic light is not itself suitable for direct illumination of a specimen.

Just as in their previous application (in combination with chromophores), the light-scattering bodies are made from a material having an index of refraction that differs greatly from the index of refraction of the matrix within which the bodies are contained. The preferred material is titanium dioxide. "Light-scattering bodies" may be more exactly defined as bodies having an index of refraction that differs from the index of refraction of the material, or matrix (commonly polymer plastic or glass) within which the bodies are contained. Titanium dioxide has, for example, an index of refraction more than twice that of glass (i.e.,  $\times 2$ ).

#### 4.4 Brightfield and Darkfield Illuminator Elements

Those embodiments of the present invention wherein the illuminator element contains fluorophores (and potentially also light-scattering bodies) may, and typically do, produce a substantially spatially uniform illumination (i.e., illumination without intensity variation detectable to the eye during microscopic observation), and, more particularly, produce a substantially uniform isotropic light illumination. The illumination so produced is substantially uniform even if the illuminator element is itself illuminated from, and by, a primary source of light that is disposed in some particular direction from the illuminator element, and that subtends but a few degrees of arc relative to the illuminator element. The fluorescent illuminator element so functions because of its many internal absorptions of light from the primary source, and because of the random directions in which the fluorescent light is emitted. Such a substantially uniform light output is called, as is the conventional terminology for condenser-based illumination, a "brightfield" illumination.

However, a fluorescent illuminator element in accordance with the present invention may also, and alternatively, be intentionally configured so as to provide "darkfield" illumination. In darkfield illumination relatively more light



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is produced at the periphery of the element -- normally at an outer annulus of a cylindrically-shaped illuminator element -- than is produced in a central region of the element -- normally at a circular central region of the cylindrically-shaped illuminator element.

The fluorescent illuminator element so configured to produce darkfield illumination is loaded with fluorophores in a higher concentration than is the case for the brightfield illuminator element. The high-concentration of fluorophores establishes a very high optical density, resulting in a self-quenching of the emitted fluorescence. This self-quenching occurs more significantly towards the center of a cylindrically, or disk, shaped illuminator element, and less significantly towards the periphery of the same element. The emitted light -- while still substantially isotropic in nature from all regions of the illuminator element -- is more intense (brighter) at a progressively higher distances radially outward from the central axis of the cylindrical illuminator element. The illumination thus produced is a direct counterpart of the darkfield illumination previously produced only by use of several separate pieces of equipment.

#### 5. Spectrally-Engineered Light

By this point it may be recognized that the illuminator elements of the present invention may contain any of (i) chromophores, whether fluorophores or pigments, and (ii) light-scattering bodies. To recapitulate, chromophores useable as the light source of the present invention may either color emissive -- i.e., luminescent chromophores and principally fluorescent fluorophores -- or color absorptive -- i.e., pigments. The light-scattering bodies useable as the light-emitting elements of the present invention, on the other hand, essentially serve to scatter light of all frequencies, and have no intrinsic color themselves. They are "white".

One easy way to think about this dichotomy, and this hierarchy, of terms and of materials is to consider the light output of the illuminator element of the present invention. The spectrum (spectra) of this light output can either be

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"engineered" internally (by use of chromophores of either type), or, alternatively, the illuminator element can simply serve to make isotropic (by multiple scattering) that light which it receives from a primary external source. In the later case the frequency or frequencies of the illuminating light are substantially determined by the external source, and not by the illuminator element itself. In either case, it is clear that the illuminator element of the present invention is a "source" of randomized distributed isotropic light (although not invariably a self-energized, intrinsic, source).

By adjustment of the numbers and types of chromophores, particularly fluorophores, and also of the light-scattering bodies that are permissively within the illuminator elements, it is possible to make illuminator elements of different colors or of multi-colors, and of differing specific brightness (i.e., luminosity) when equivalently excited (by an external primary radiation source). Either part of this capacity of the illuminator elements of the present invention is called "spectrally-engineered light". Microscopists have traditionally paid much attention to (i) the staining of specimens, and (ii) the filtering of the light by which the specimens are observed, in order that the contrast, and the resolution (at narrow bandwidth), of microscopic observations and/or photomicrography may be improved. The present invention now permits that attention may be paid to the illumination spectrum (spectra) of the illuminator elements for the same purposes.

#### 6. Use and Utility of the Illuminator Elements

An illuminator element of the present invention is not only a source of isotropic light, its close placement to the specimen serves to substantially isotopically illuminate the specimen over at least the area of observation, and normally over the entire specimen. Thus the illumination is not only isotropic -- meaning that it proceeds from no preferred direction or directions, and instead proceeds equally from all directions in common -- at the face of the illuminator element where it is produced, but is substantially isotropic upon the surface of the specimen where the illumination is used.

A illuminator element commonly has at least one flat face, which face is more commonly made optically flat. This flat face is commonly placed proximately to or, more commonly upon and in direct contact with, either a specimen, a microscope slide, or a coverslip. The specimen, commonly mounted upon a microscope slide, is also commonly flat (to the limits of being sectioned, if a sectioned specimen). Although two relatively flat surfaces are most commonly placed into direct contact, it should be understood that neither (i) flatness nor (ii) contact is required. It is sufficient simply that the illuminator element and its light-emitting surfaces, howsoever they be contoured, should be located sufficiently closely to the specimen so that at least the area of the specimen to be microscopically observed is nearly completely bathed in substantially isotropic light over a considerable solid angle that is preferably nearly two pi ( $2\pi$ ) steradians (i.e., the solid angle subtended by a hemisphere at the centroid of a sphere).

The isotropic illumination from the illuminator element bathes the specimen in light from all directions of nearly an entire imaginary hemisphere, thus (i) illuminating the specimen without shadows, (ii) reducing glare from the microscope optics, (iii) eliminating projection of out-of-focus elements onto the image plane, and (iv) completely filling the objective lens of the microscope with a homogeneously bright field. This specimen illumination directly fulfills many of the requirements, and is analogous to satisfying certain remaining requirements, of Koehler illumination. It is not precise to say that the illumination provided by the illuminator element of the present invention is precisely Koehler illumination because Koehler dealt with the requirements of the different, traditional, illumination of a specimen by use of condensers.

However, an appreciation of the utility of the present invention may be gained when it is realized that the quality of microscopic images obtainable with illuminator elements of the present invention -- all of which are commonly producible in quantity for only a few dollars each -- may be favorably compared to the best images obtainable from entire sets of the best

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available condensers costing in the range of \$1,000-\$5,000 U.S. each condenser. The quality of the magnified images realizable with any of the several different embodiments of illuminator elements in accordance with the present invention is -- if inferior to the best condensers at all -- only but very slightly so.

Moreover, it will be appreciated that there is no great skill or labor involved in simply placing the illuminator element of the present invention proximate to the specimen.

Moreover, it will be recalled that each illuminator element in accordance with the present invention produces a precisely predetermined light spectrum or spectra by which a specimen may be observed. The typically yellowish light of even a high-quality quartz-halogen microscope illuminator, or even the considerably worse and spectrally impure light of a common incandescent light bulb, may both be readily transformed by an appropriate illuminator element of the present invention into virtually any desired color or colors of light. This is called "spectrally-engineered light" in the present invention. Spectrally-engineered light can be extremely useful for resolution of a feature or features of the specimen under observation. An external light source may be in particular transformed into synthesized pseudo-white lights that are superbly matched to any of the human eye, video camera tubes, charge-coupled devices, or photographic film.

Moreover, it will be recalled that illuminator elements in accordance with the present invention can produce either brightfield or darkfield illumination.

#### 7. Variations of the Illuminator elements

Illuminator elements in accordance with the present invention -- based upon light-manipulating chemicals contained within a stable matrix -- are commonly used to channel and transform light from an external light source. These illuminators devices can, however, use internal chemiluminescent or radioluminescent light sources.

Extending on the concept of an illuminator element that internally produces, and not merely transforms, light, an

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illuminator element may be constructed in the form of a more complex, non-unitary, element. Such an illuminator is commonly externally powered, normally by electricity, to produce the isotropic light alone and by itself. For example, an illuminator element could be based on an electro-fluorescent light source -- commonly an electrically or magnetically excited fluorescent light, or sheet light, or other electrically or magnetically powered source of isotropic light -- that is placed so close to the specimen so as to bathe it in isotropic light.

Accordingly, it should be realized that, once the immense utility to microscopy of the (substantially) isotropic specimen illumination taught by the present invention is recognized, there are a great number of diverse ways to produce such illumination. All natures and types of sources of isotropic light are therefore within the scope of the present invention.

Moreover, isotropic illumination of a specimen at electromagnetic frequencies other than those of light radiation may sometimes be desirable, possible and appropriate for microscopes performing observations at, and in, these other electromagnetic frequencies. Accordingly, the present invention should be broadly perceived as a new approach to specimen illumination during (all types of) microscopy; namely to illuminate the specimen isotopically over nearly a hemisphere as opposed to directionally on-axis, and by a universal illuminator element as opposed to the use of a condenser matched to the numerical aperture of the microscope's objective lens.

These and other aspects and attributes of the present invention will become increasingly clear upon reference to the following drawings and accompanying specification.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a diagram showing the cone of illumination of a prior art microscope where the substage condenser and the diaphragm are properly adjusted so that the cone of illumination completely fills the aperture of the microscope objective.

Figure 2 is a diagram showing image-forming ray paths that are traced from ends of lamp filament of a prior art microscope

where conjugate foci are at the field diaphragm, specimen plane, intermediate image plane (entrance pupil of eyepiece) and, with a camera in place, the film plane.

Figure 3, consisting of Figures 3a through 3c, shows prior art condensers respectively of the abbe, aplanatic and aplanatic achromatic types.

Figure 4, consisting of Figures 4a through 4c, shows the cones of light respectively transmitted by the prior art abbe, aplanatic and aplanatic achromatic condensers previously seen in Figures 3a through 3c.

Figure 5 shows a prior art optical train adjusted for Koehler illumination, particularly illustrating the conjugate aperture planes,  $S_1$ - $S_4$ .

Figure 6 shows the prior art optical train previously seen in Figure 5 adjusted for Koehler illumination, particularly illustrating the conjugate field planes  $I_1$ - $I_2$ .

Figure 7a is diagram of brightfield microscopy where central light passes through, around and/or surrounds the specimen under microscopic observation.

Figure 7b is diagram of darkfield microscopy where central light is blocked and where only oblique rays strike the specimen under microscopic observation.

Figure 7c is a ray trace diagram of darkfield illumination showing that, when all un-deviated rays of the zero'th order have been blocked, oblique rays, now diffracted by the specimen, proceed on to the image plane and form an image of the specimen.

Figure 8 is an optical schematic diagram of a prior art transmission microscope using (i) a condenser and (ii) a light source.

Figure 9 is an optical schematic diagram of the transmission microscope, including the light source, previously seen in Figure 8 now in use with an illuminator element of the present invention; the condenser previously seen in Figure 8 now being completely eliminated.

Figure 10 is an optical schematic diagram of a prior art epi-fluorescent microscope using (i) two condensers and (ii) a light source.

Figure 11 is an optical schematic diagram of the epi-fluorescent microscope, including the light source, previously seen in Figure 10 now in use with an illuminator element of the present invention; one condenser previously seen in Figure 10 now being completely eliminated.

Figure 12, consisting of Figures 12a through 12o, are diagrammatic representations, shown at approximately twice real scale, of various shapes and forms of illuminator elements in accordance with the present invention.

Figure 13, consisting of Figure 13a and Figure 13b, are diagrams of a fluorescent illuminator element of the present invention having the substantial form of Figure 12h held within two different variants of an appliance that also holds a primary source of light, particularly in the form of a pen light, that serves to excite the fluorescence of the illuminator element.

Figure 14 is a diagram of a prior art microscope iris that is usefully employed in combination with an illuminator element of the present invention, including but not limited to that particular fluorescent illuminator element which is held within that particular appliance that is shown in Figure 13b.

Figure 15 is a graph showing an exemplary fluorescent spectra of pseudo-white light produced by three particular fluorophores that are used in an exemplary illuminator element in accordance with the present invention.

Figure 16a is a diagrammatic representation of a brightfield illuminator element in accordance with the present invention, the brightfield illuminator element producing a relatively even magnitude of illumination at its top surface, which surface is positionally disposed towards the specimen under observation.

Figure 16b is a diagrammatic representation of a darkfield illuminator element in accordance with the present invention, the darkfield illuminator element producing a relatively more intense illumination at the peripheral, as opposed to the central, regions of its top surface, which top surface is positionally disposed towards the specimen under observation.

Tables 17, consisting of Table 17a through Table 17c, is a table of fluorophores, also called fluorochrome chemicals, that

are generally suitable for use in construction of illuminator elements in accordance with the present invention.

#### DESCRIPTION OF THE PREFERRED EMBODIMENT

##### 1. Principles of Prior Art Illumination Relative to The 5 Illuminator Elements of the Present Invention

As discussed in the subsection 2.1 General Background, of the section 2. Description of the Prior Art, of the BACKGROUND OF THE INVENTION portion of this specification, the cone of illumination of a prior art microscope is diagrammatically shown in Figure 1. Similarly, Figure 2 is a diagram more completely showing the image-forming ray paths that are traced from ends of lamp filament of a prior art microscope.

Also as previously discussed. prior art condensers of the abbe, aplanatic and aplanatic achromatic types are respectively shown in Figures 3a through 3c. The cones of light transmitted by the prior art abbe, aplanatic and aplanatic achromatic condensers seen in Figures 3a through 3c are respectively shown in Figures 4a through 4c.

Also as previously discussed, the various lenses of a prior art microscope are arranged as shown in Figures 5 and 6 for Koehler illumination. Adjustments of the prior art lenses give rise to the two sets of optical paths and the image planes represented in Figures 5 and 6. Together, the two sets of optical paths and the image planes characterize Koehler illumination.

Likewise, a ray trace diagram of prior art brightfield microscopy where central light passes through, around and/or surrounds the specimen under microscopic observation is shown on Figure 7b. A ray trace diagram of darkfield microscopy where central light is blocked and where only oblique rays strike the specimen under microscopic observation is shown in Figure 7b. Finally, a ray trace diagram of darkfield illumination showing that when all a un-deviated rays of the zero'th order have been blocked then oblique rays, now diffracted by the specimen proceed on to the image plane and form an image of the specimen, is shown in Figure 7c.



A ray trace diagram of a prior art transmission microscope in use for observing a specimen is shown in Figure 8, and an equivalent ray trace diagram for a prior art epi-fluorescent microscope is shown in Figure 10. The diagrams of Figures 8 and 10 are particularly intended to be juxtaposed relative to, and compared with, the respectively corresponding ray trace diagrams of Figures 9 and 11 showing illuminator elements in accordance with the present invention in operational use.

The present invention, replacing as it does the condenser of a microscope, will be seen to derive the essential optical characteristics of Koehler illumination, and of brightfield and darkfield illumination, without the expense, or difficulty, of adjusting condensers to realize the optical paths variously shown in Figures 1-8 and 10.

## 2. Function and Usage of Illuminator Elements in Accordance With the Present Invention

An illuminator element in accordance with the present invention is made from (i) spatially distributed light sources carried in or upon, and normally within, (ii) a stable matrix that is suitably sized and shaped so as to suitably be positioned closely proximate a specimen under observation.

### 2.1 Isotropic Illumination

The matrix, and its typically contained spatially distributed light sources, must be sized and shaped so as to suitably be positioned closely proximate to the specimen under observation. Only in this manner may the specimen be substantially isotropically illuminated by the light source. The positional relationship between the specimen, the isotropic light source (the illuminator element in accordance with the present invention) and the objective lens of the microscope is this: the specimen is situated between the isotropic light source (the illuminator element) and the objective lens. An illuminator elements in accordance with the present invention so positioned is shown in each of Figures 9 and 11, where it is identified simply as an "ILLUMINATOR".

The "substantial isotropic illumination" of the specimen is normally accomplished by placing the illuminator element in

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direct contact with the specimen, or at least in contact with a microscope slide on which the specimen is mounted (normally at the side towards the objective lens of the microscope and spaced apart from the illuminator element, but permissively on the opposite side). The illuminator element may, however, stand off a bit from the specimen, normally by only a fraction of a millimeter, if so desired. This slight distance of separation may serve, for example, to reduce contact contamination between the microscope slide (or the specimen itself) and the illuminator element.

However, image quality degrades with increasing separation between the specimen and the illuminator element. Conversely, image quality, and particularly resolution, improves with the proximity of the specimen to the illuminator element. This relationship is due to the fact that the intensity, angle and uniformity of plane-wave illumination from an isotropic light source comprised of spatially distributed emitters of light is optimal closest to the source. In fact, because the source transfer function of an illuminator element in accordance with the present invention is, unlike that of a condenser, independent of frequency, an illuminator element of the present invention can, theoretically, be superior to any prior art light-source-and-condenser in the resolution of small, closely-spaced objects!

As the distance between specimen and source increases the homogeneity, and plane-wave character, of the illuminating light progressively degrades at the plane of the sample. At some increasingly large distance, typically several millimeters, from the specimen, the illuminator element will cease to provide illumination of sufficient quality so as to form a reasonable image. Although mis-adjustment of the positioning of the illuminator element by failing to place it in sufficient proximity to the specimen is unlikely, it is to be noted that such mis-adjustment reveals itself clearly and unambiguously in the observed image, and is neither latent nor poorly detectable as has been the mismatch, or mis-adjustment, of a prior art light-source-and-condenser.

Additionally, and as will be seen from the many permissible

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physical shapes of the illuminator elements -- examples of which are shown in Figure 12 --, an illuminator element typically presents a flat, and more typically an optically flat, surface disposed towards the specimen. However, such surface need  
5 neither be flat nor even smooth. Accordingly, it will be recognized that for some shapes of illuminators, and rarely also for some relatively thick specimens, it may be impossible to place a (flat) illuminator element uniformly tight against a (substantially flat) specimen. This separated, or spaced  
10 parallel, placement of the illuminator element and specimen does not unduly interfere with the operation of the illuminator element. It will, however, be understood that, in the spirit of the invention, that the "substantial isotropic illumination of the specimen" can only be obtained from an illuminator element -  
15 - itself an isotropic light emitter -- that is positioned closely proximate to the specimen.

Meanwhile, an isotropic light source is, by dictionary definition, one that lacks predetermined axes, presenting equal characteristics along all axes and in all directions. The sun,  
20 and a common fluorescent light bulb, are both isotropic light sources. In order for illumination to be isotropic it must come from all directions without distinction or differentiation. Unless contained inside a luminous sphere, only a physically small body can approximate being perfectly isotopically  
25 illuminated, and then only by close proximity to an isotropic light source.

A portion of a microscope slide, and all or most of a thin specimen mounted thereon, may be, and is, in accordance with the present invention "substantially", or "approximately",  
30 isotopically illuminated. "Substantially isotopically illuminated" as used in this specification means, when referring to the illumination of a specimen, that a specimen (and any microscope slide upon which the specimen may be mounted) are positioned so close to an isotropic light source that no  
35 shadow(s) are visible -- including upon the sides, or shoulders, of the specimen -- when the specimen is viewed through the microscope.

The illuminator element is a source of light extending over four pi ( $4\pi$ ) steradians, or a complete sphere. Because this light source is large and close relative to the specimen region of interest (i.e., the specimen region under microscopic observation), straight lines, or rays, can be traced between the specimen and nearly a full two pi ( $2\pi$ ) steradians, or a complete hemisphere of solid angle, of the illuminator element light source. Due to a typical difference in refractive index between the illuminator element and the specimen (or the microscope slide, or the cover slip), so much of the of the light illumination from the illuminator element as exceeds the critical angle will not pass out from the illuminator element and into the specimen. This light that is reflected at an internal interface boundary, or surface, of the illuminator element is hypothesized to result in a "pooling" of light in the illuminator element at its surfaces, including at that one of its surfaces that interfaces to the specimen.

This hypothesized operation may contribute to the brightness of the illuminator elements, which brightness is generally eminently satisfactory for microscopy even when the illuminator elements are themselves externally illuminated, as will be seen, with a common pen-light-type flashlight having no great brightness. It should, however, be understood that the "pooling" of light within the illuminator elements is only a hypothesis, and that this and much else of the theory and the ascribed attributes of the present invention -- particularly including the apparent production of isotropic light by the illuminator elements and the hypothesized mechanisms therefore -- are discussed only so that the invention may be better considered and understood, and not so as to be limiting of Applicants' invention as taught and claimed.

The illumination of a specimen that is obtained by use of an illuminator element in accordance with the present invention is clearly much different, whatsoever the theory behind the generation of this illumination and howsoever this illumination is described, than is the directional light illumination of a specimen within the prior art. The lengthy discussion of

isotropic illumination in this sub-section has been necessitated by the limitations of words to describe the exact nature of the illumination transpiring at all points of interest, and in order to make as definitive as is possible the precise type of illumination that is, to the best understanding of the inventors, both produced and used in the conduct of microscopy in accordance with the present invention.

## 2.1 The Production and Use of Isotropic Illumination During Microscopy

In accordance with the present invention, one, preferred, source of spatially isotropic light is a fluorescent material contained within a matrix. A fluorescent material is, by dictionary definition, capable emitting electromagnetic radiation resulting from and during the absorption of excitation radiation from an external source thereof.

In accordance with the present invention, the fluorescent material is preferably contained in a polymer matrix in the shape of, typically, a small disk, cylinder or cube. The matrix is of size and shape so as to suitably be positioned closely proximate to a specimen -- which specimen is normally (but not necessarily) mounted upon a microscope slide -- that is to be observed by and through a microscope. So positioned, upon and during absorption of the excitation radiation from the external source by the element's fluorescent material, thus fluorescent material illuminates the specimen with emitted electromagnetic radiation in the form of fluorescent light. This fluorescent radiation is isotropic: it is emitted from the fluorescent material everywhere within the element in random directions, and it exits the faces of the element, including through the face opposite the specimen, in a uniform, wide-angle distribution. The fluorescent radiation is suitably intense so as to permit observation of the specimen by, and with, the microscope.

The element is commonly adapted for use with an optical microscope. Because it is positioned closely proximate to the specimen that it serves to illuminate with light, its illumination of the specimen is (i) automatically aligned, and (ii) automatically matched to the numerical aperture of the

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microscope's objective lens, with (iii) many of the characteristics of Koehler illumination being achieved.

Accordingly, an illuminator in accordance with the present invention completely supplants the condenser of an optical microscope, and completely obviates the labor of adjusting the light source and the condenser of the microscope.

The fluorescent material is preferably in the form of one or more fluorescent dyes each of which has an associated emission spectrum. The fluorescent material is more preferably in the form of several different fluorescent dyes. Each dye is responsive to the same radiation from a single external radiation source for emitting light at an associated emission spectrum. The several dyes collectively produce an emission spectra that is preferably of a pseudo-white, "engineered" light.

The fluorescent material is preferably further combined in the matrix with a scattering material that serves to scatter and to disperse the light emitted by the fluorescent dyes.

The matrix is preferably in the form of a polymer plastic or glass body, typically in the shape of a coin or disk, or else an elongate parallelepiped body similar to a microscope slide. At least that surface of the element's body that serves to transmit light onto the adjacent specimen is normally optically polished so that it does not interfere with, nor diminish, the isotropy of the light. This surface is typically the circular face of the coin- or cylinder-shaped body, or a face of a cube or a parallelepiped body. The opposite face is also typically polished for (i) aesthetic symmetry, (ii) to permit visual, non-microscopic, viewing of the specimen completely thorough the body of such elements as are translucent or transparent, and/or (iii) to permit external excitation radiation (should it be delivered into the element through this opposite face and it need not be so delivered even in part through this face) to be, if partially reflected as well as being refracted into the element, so reflected at a determinable regular angle. In other words, in some microscopy and microscopic photography, it is not desired to have extraneous light in the room, for example a laboratory, where the microscope is situated; no matter how irrelevant such

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light may be to the specimen illumination. The illuminator elements of the present invention are normally of regular shape not because they have to be, but because the optical properties of such regularly shaped elements may be more readily understood and controlled by the user.

The preferred embodiment of an illuminator element in accordance with the present invention for use with an optical microscope is both straightforward of construction and relatively economical, costing only a few United States dollars each, circa 1994. It is accordingly difficult to see, at first blush, how such a compact and inexpensive item could adequately suffice to completely supplant microscope condensers costing hundreds and thousands of dollars.

3. Use of Illuminator Elements in Accordance with the Present Invention With, For Example, Both Transmission and Epi-Fluorescent Microscopes

An illuminator element in accordance with the present invention in use with an existing prior art transmission optical microscope is shown in Figure 9. An illuminator element in accordance with the present invention -- which may or may not be the selfsame identical illuminator element as is shown in Figure 9 -- in use with an existing prior art epi-fluorescent optical microscope is shown in Figure 11. It may be immediately noticed that the condensers shown in Figures 8 and 10 are eliminated. The light source of Figure 9 may, or may not be, the light source shown in Figure 10. The existing epi-fluorescent light source (which is not a source of fluorescent light but is only a bright, typically halogen or xenon or mercury, light source) shown in the prior art microscope of Figure 10 is intended to be that light source that is also shown in Figure 11 -- but this need not be the case.

An illuminator element in accordance with the present invention most particularly does not have to be used with these particular microscopes, and is suitable for general use with all optical and optical-type microscopes (i.e., microscopes of ultraviolet and infrared radiation). Indeed, usage with microscope having the particular simplified optical trains shown

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in Figures 9 and 11 may well be but a fraction of all uses. However, just as the illuminator element of the present invention had its genesis in the context of conventional epi-fluorescent microscopy (as explained in the preceding section), it is useful to think about the light paths of conventional standard and epi-fluorescent microscopes, shown in Figures 8-11, when first thinking about the nature, qualities, source and/or path of the electromagnetic radiation -- typically light radiation -- that serves to excite the predominant (non self-energized), and particularly the fluorescent and/or scattering, embodiments of the illuminator element.

The basic (i) placement, (ii) energizing by an external primary light source, and (iii) use of the isotropic light illumination produced by the illuminator elements is straightforwardly shown in both Figures 9 and 11. However, a first concept to be understood is that restrictions on the (i) placement of the external, primary, light source relative to both the illuminator element and the microscope are very liberal, and almost non-existent. It is sufficient only that the external light source be placed so as to (i) shine sufficient light onto the illuminator element while (ii) avoiding putting extraneous light into the optical path of the microscope in any manner by which it will become detectable at the microscope's eyepiece (reference Figures 8 and 9 for the eyepiece). The placements of the primary light source in both Figures 9 and 11 satisfy this criteria. However, so also does the placement of the flashlight light source in each of Figures 13a and 13b -- regardless of what type of optical microscope these flashlights and associated illuminator elements (called ILLUMINATORS in Figures 13a and 13b) are used with.

There are also certain criteria -- not visible in nor apparent form Figures 9 and 11 -- for the appropriate frequency(ies) of the (i) energizing, and (ii) illuminating (light) radiation. Consider that one major type of illuminator element in accordance with the present invention consists essentially of fluorescent material. This is a material that, by dictionary definition, emits electromagnetic radiation at a



first frequency or frequencies in response to, and during, the absorption of radiation at a different second frequency or frequencies arising from some other source. Whatsoever the type of the microscope with which this type of illuminator element is used, observation is performed at, and with, only the radiation of the first frequency or frequencies: radiation of the second frequency or frequencies is both unused for, and must be non-conflicting with, this observation. The microscope, and its objective, is suitable, by definition, to focus the first frequency or frequencies. Sometimes, in embodiments of the present invention particularly for use with an epi-fluorescent microscope where the excitation radiation comes through the microscope prior to exciting the fluorescent material of the illuminator element, the microscope, or at least its objective lens, must also suffice to focus the radiation of the second frequency or frequencies.

Consider an example to illustrate this last point: an illuminator element of the present invention may be excited by radiation passing in the illumination path of an epi-fluorescent optical microscope. The fluorescent material of the illuminator element would produce visible light first-frequency radiation in response to absorption of second-frequency radiation. However, this second-frequency radiation need not be visible, and could be invisible. The produced visible light radiation is, by the very definition of an optical microscope, of a frequency range suitable for illuminating a specimen that is viewed through the microscope. Importantly, the invisible radiation which travels through the microscope and to which the fluorescent material is responsive is also of a frequency, or range of frequencies, that is (are) suitable to be manipulated by the optical microscope, or at least by the microscope's objective lens. In other words, in the case of an illuminator element usable with an optical microscope, the invisible radiation is still light radiation, although perhaps outside the range of detection by the eye, film, or whatever currently serving as the detector of the microscope.

The illuminator element for use with an epi-fluorescent microscope is, as is always the case, suitably sized and shaped

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so as to be positioned closely proximate to a specimen under observation. So positioned, radiation of a second frequency will, after optionally passing in an optical path through the objective of the microscope and through the specimen, impinge upon the fluorescent material. It is therein converted to radiation of the first frequency, by which first-frequency radiation the specimen is observed.

Of course, and illuminator element in accordance with the present invention is usable in other than an epi-fluorescent microscope -- witness the transmission microscope of Figure 9. A concept to be grasped is simply that the frequency(ies) of the excitation radiation (light) may, or may not, equal, or substantially equal, in accordance with the physics of fluorescence, the frequency(ies) of the illumination radiation (light). This does not show up on the Figures, where all rays look the same. It may, however, be a consideration for some applications of illuminator elements in accordance with the present invention. For example, flash excitation of an illuminator element with radiation outside a sensitivity range of a certain film might not require either shielding or shuttering of the film in a darkened laboratory, presenting the possibility of building an image -- possibly of a dynamic occurrence -- by multiple exposures.

Another, crucial, concept to grasp is that, for all embodiments of the invention -- including those where the second-frequency radiation passes through the objective of an epi-fluorescent microscope before it is converted by the fluorescent material of the illuminator element into the first-frequency radiation by which the specimen is (microscopically) observed -- the stimulated fluorescence, or first-frequency radiation, completely fills the aperture of the microscope objective! In the epi-fluorescent microscope configuration of Figure 11 the optical characteristics of the objective are effectively conferred upon the illuminator element, making automatic both (i) alignment and (ii) matching of the numerical aperture of the objective and the illuminator. In any configuration -- whether those of Figure 9 or Figure 11 or otherwise -- Koehler-like

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conditions (or their equivalent in non-light microscopy) are achieved.

Accordingly, it is somewhat crude, and potentially limiting, to think of illuminator elements in accordance with the present invention as simply "light sources", of "glowing bodies", or some other appellation which, while true, may fail to capture the sophistication, and elegance, of the real thing. Instead of thinking imprecisely, a due consideration of what is really going on by use of the illuminator elements of the present invention will suffice to provide one definition of such elements. The illuminator elements have and present a great multiplicity of light sources randomly distributed in space. This great multiplicity of light sources produces a corresponding great multiplicity of light wave fronts in random directions. Some ones of these random-direction wave fronts as arise from the great multiplicity of spatially-randomly-distributed light sources intercept the specimen in its position proximately located to the illuminator, therein serving to illuminate the specimen evenly with a substantially isotropic light.

Thus when other, more universal, embodiments of the illuminator element of the present invention are hereinafter discussed in detail, it should not be presumed that, because the range of radiation to which each such device is sensitive and which each such device produces, is preset, that either or both of these ranges are somehow limited, and must be of some preconceived nature such as "light" or "visible light". Indeed, it will be feature of the present invention, as hereinafter explained, that it is designed in various embodiments to produce, from selectively preset input radiation stimulus (stimuli), selectively preset, "engineered", radiation outputs.

#### 4. General Construction of an Illuminator Element in Accordance with the Present Invention

Consider the typical size and shape of an illuminator element usable with an optical microscope. A typical illuminator element is formed in the shape of a disk, normally of a size about 10 mm diameter and 2-5 mm in thickness (i.e., smaller in diameter than a United States dime, and of roughly the same

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thickness as a stack of 2-5 dimes). It is typically so formed by process of molding, normally of liquid polymer plastic or glass. Such an illuminator element is illustrated in Figure 12h.

O.e, major, type of the typically-shaped, disk, illuminator element consists essentially of (i) one or more fluorescent chemicals (alternatively called "fluorochromes", or "fluorophores") as its active, light-producing agents plus, potentially and preferably, (ii) light-scattering particles of one or more types, held stably in a polymer matrix. Each fluorescent chemical has an associated fluorescence spectrum. Several fluorescent chemicals may collectively produce a fluorescence spectra that is substantially a pseudo-white light. The colored or pseudo-white light(s) produced by the illuminator element serve to illuminate the specimen. The fluorescent light output of these chemicals is the reason that light rays are illustrated to emanate from the illuminator element illustrated in Figure 12h.

In one particular embodiment of this type of illuminator element the fluorescent chemicals are three in number, each of which produces one of three primary colors. The typically several fluorescent chemicals are normally all sensitive to a single radiation excitation, typically light excitation that is substantially of a single frequency. In actual use in microscopy, the fluorescent chemicals are typically so excited with a single frequency of radiation. The exciting radiation may be a visible or an invisible light, and if invisible is normally ultraviolet light.

The principles of the present invention clearly extend to microscopes, and microscopy, using other than visible light. In accordance with the present invention, the frequency range at which observations will be performed in, and with, a particular type of microscope must first be identified. Next one of more chemicals -- each generally to be called a fluorescent chemical where such term is understood not to be limited to light radiation -- that is sensitive to an excitation radiation to produce light radiation within the observational frequency range must be identified. If the illuminator element is to be used

with an epi-fluorescent microscope as is diagrammed in Figure 11, then the frequency of the excitation radiation used to excite the fluorescent chemical(s) of the illuminator element is typically (i) outside the observational frequency range of the microscope, but (ii) suitably focused by the objective of the particular epi-fluorescent microscope.

In operational use, the illuminator element with its contained fluorescent chemical(s) in all cases located next to the specimen, with the specimen between the illuminator element (and its contained fluorescent chemical(s)) and the objective of the microscope. The radiation to which the fluorescent chemical(s) is (are) sensitive is directed to intercept this (these) fluorescent chemical(s) at its (their) location(s) within the illuminator element. The stimulated radiation emission of the fluorescent chemical(s) will illuminate the specimen, and is then focused by the objective lens.

5. Detail Construction of an Illuminator Element in Accordance with the Present Invention

In the construction of one preferred embodiment of an illuminator element in accordance with the present invention, fluorescent molecules (fluorophores) are combined with two copolymers to produce a fluorophore-polymer unit that releases light upon exposure to a primary radiation, normally a light radiation, source. The emitted light illuminates the specimen - avoiding the use of all microscope condensers and condenser light sources. The illuminator element of the present invention is simple to use, cheap, serves to eliminate most image artifacts, provides resolution comparable to standard microscopy, and provides automatic numerical aperture matching to all objectives.

The illuminator element (i) is made from a material that is capable of randomizing incident radiation, if not also converting such radiation from a first to a second frequency, and (ii) is positioned closely proximate to a specimen under observation by a microscope. If the illuminator element so serves to convert the incident radiation, then it is the second frequency radiation by which the specimen is observed.

The illuminator element is typically in the shape and size of a small disk. The element is normally made from "material" consisting essentially of one or more fluorescent compounds held stably in a polymer matrix. The positioning of the illuminator element "closely proximate" the specimen under observation typically serves to place the illuminator element in direct contact with the specimen. The "microscope" is typically an optical microscope.

The illuminator element thus serves as an "illuminator" of the specimen, which is why it is so called. The illuminator element preferably illuminates the specimen with (i) visible fluorescent light, and more particularly with each of three primary colors of visible light (normally red, green and blue colors), generated in response to (ii) an appropriate, typically invisible, single-frequency radiation excitation. However, the illuminator element need not illuminate the specimen with visible light, nor with any particular color(s) thereof -- it being understood that the radiation frequency-shifting principles of the invention are applicable to specimen illumination in other than visible or colored light.

A major advantage of the illuminator element in accordance with the present invention is its ability to produce one or more predetermined color(s) (i.e., radiation frequencies) at which a specimen is illuminated from, and by use of, one only primary illumination source. Color(s) selection(s) is (are) made by choosing appropriate fluorescent compounds having the desired fluorescence spectra.

A major advantage of specimen illumination in accordance with the invention is that when the specimen is in focus to the objective of a microscope then so also will a Koehler-like illumination (Koehler, 1893; Dempster, 1944) illumination of the specimen be obtained. The normally difficult and time consuming task of adjusting a condenser-objective pair of a microscope illumination stage in order to obtain (i) Koehler illumination and (ii) matched numerical apertures (N.A.) is completely eliminated!

## 6. Principles of Operation

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Fluorescent light is isotopically emitted from all the surfaces of the illuminator element, which isotropic light emission is intended to be represented for one surface only -- the surface facing the specimen -- in Figures 9 and 11 by the arrows, or vectors of light, that proceed from the illuminator onto the specimen in, and from, multiple random directions. The illuminator element forms an emitted, isotropic, secondary light beam that illuminates the specimen for wide field image formation. The isotropic secondary illumination cone completely fills the aperture of the objective lens. Thus, the optical characteristics of the objective lens are essentially conferred upon the illuminator element -- resulting in automatic alignment and objective-illuminator numerical aperture (N.A.) matching. In addition, substantial Koehler, or near-Koehler, conditions of illumination (discussed in greater detail in section 9., following) are achieved. This is particularly easy to see in the epi-illumination microscope configuration of Figure 11 since the source #2 excitation and the illuminator element emission are geometrically coincident.

The illuminator element does not care how, nor from where, it receives the primary excitation radiation. As may be seen in Figure 13, common incandescent light sources held by relatively crude holders in position so as to shine upon the illuminator elements will suffice to provide this primary excitation radiation. These common external light sources crudely held generally permit the illuminator element to produce its secondary fluorescent light emission in quite as satisfactory a manner as do primary light sources that are (i) brighter, (ii) spectrally more pure, (iii) better collimated, (iv) more expensive, or (v) superior in any manner whatsoever.

However, it must be admitted, the conventional epi-fluorescent microscope, in particular, provides in its pre-existing structure a very simple, and convenient, way to provide primary excitation light to the illuminator element. Moreover, the characteristics of this light can readily be varied. This can be useful in the development of illuminator elements (a task normally undertaken by the manufacturer, and not the user,



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thereof). For example, a set of filters allows excitation light to pass through the illuminator and prevent image plane exposure to source #2. Due to the tremendous variety of fluorophores and filters available for fluorescent microscopy, it is possible to  
5 create almost any combination of excitation/illumination pair.

In order to produce a pseudo-white-light illumination and full color images, (i) a 365 nm wavelength primary excitation light source has been preferred for use with (ii) preferred illuminator elements in accordance with the present invention  
10 having blue, green and red-emitting fluorophores embedded in the polymer matrix is preferred. Figure 15 illustrates the fluorescence emission spectra of a first preferred embodiment of an illuminator element that is (i) so illuminated, and (ii) that contains each of these three fluorescent chemicals. The number  
15 of possible embodiments of the illuminator elements will turn out to be almost endless, as is suggested by the attached Table 17. It is sufficient to understand that light emitted from an illuminator element which light may appear as white to the eye is, in fact, a sum of several distinct colored emission spectrum.

20 All fluorophores are stable and have high fluorescence quantum yields, permitting the use of low-intensity excitation sources. When paired with low power objectives, a pinhole or field diaphragm placed between the illuminator and the specimen increases image contrast. The use of an adjustable iris between  
25 an illuminator element, such as happens to be held along with a flashlight in a holding appliance in Figure 13b, is shown in Figure 14.

Figure 12, consisting of Figures 12a through 12o, show exemplary physical forms and shapes of several different  
30 illuminator elements. The illuminator elements of Figures 12a and 12h "sparkle" by virtue of their fluorescent light emission under normal ambient room lighting. Some of the illuminators are substantially transparent to visible light, others are translucent colored bodies, some are translucent, and still  
35 others are opalescent. A illuminator element may marked with indicia -- including by embossing or molding -- on its top (as in Figures 12f, 12m, 12n and 12o) or side surfaces (as in Figure

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12l) without deleterious effect on its function. The illuminator elements shown in Figures 12m thorough 12o are respectively three-dimensionally contoured with the English letters "R", "G" and "B" as might denote, for example, the respective provision of Red, Green and Blue light by these illuminator elements 12m-12o.

## 7. Construction of the Preferred Embodiments

### 7.1 Materials

The major, fluorescent-light-emitting, embodiment of an illuminator in accordance with the is present invention is preferably constructed from (i) a polymer host and (ii) fluorescent dyes. The polymer host is preferably copolymer of polymethylmethacrylate (alternatively called methylmethacrylate) and poly-2-hydroxymethyl methacrylate (alternatively called 2-hydroxymethyl methacrylate) in proportion 3:1 by volume, and more precisely in a proportion of 70 ml. to 30 ml. by volume.

The preferred fluorescent dyes in support of black and white photomicrography is rhodamine 6G. Rhodamine 6G has a fluorescent absorbance maxima  $\lambda_{abs}(max) = 528$  nm, and a fluorescent emission maxima  $\lambda_n(max) = 555$  nm). The amount of rhodamine 6G used is preferably that amount as will establish an optical density (O.D.) of the rhodamine 6G at an excitation wavelength 365 nm in the mixture to be within a range of 0.3-0.5 per 1 cm. (Optical density is the standard measure of quantifying an amount of an optically active substance such as a fluorophore.)

For pseudo-white light illumination in support of color photography, a mixture of four fluorophores is preferable:

A first fluorophore is coumarin 152; added to a predetermined optical density in the mixture. Coumarin 152 has a fluorescent absorbance maxima  $\lambda_{abs}(max) = 394$  nm, and a fluorescent emission maxima  $\lambda_n(max) = 496$  nm.

A second fluorophore is rhodamine 110 --  $\lambda_{abs}(max) = 498$  nm,  $\lambda_n(max) = 520$  nm. Rhodamine 110 is also added to a predetermined optical density in the mixture, and normally to the same optical density as the coumarin 152.

A third fluorophore is sulforhodamine 640 --  $\lambda_{abs}(max) = 578$  nm,  $\lambda_n(max) = 605$  nm.. It is again added to a predetermined

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optical density in the mixture, and normally to the same optical density as the coumarin 152 and the rhodamine 110.

A fourth, and final, fluorophore is the same rhodamine 6G ( $\lambda_{\text{abs}}(\text{max}) = 528 \text{ nm}$ ,  $\lambda_{\text{em}}(\text{max}) = 555 \text{ nm}$ .) as was used in the  
5 monochromatic illuminator (see above). It is again added to a predetermined optical density in the mixture, and normally to the same optical density as is each of the coumarin 152, the rhodamine 110, and the sulforhodamine 640.

For example, and by way of the creation of the particular  
10 embodiment of an illuminator element, the optical density (O.D.) of Coumarin 152 at the standard preferred excitation wavelength (365 nm) was set to 0.3 per 1 cm, and the concentrations of all other dyes were adjusted to get the same fluorescence intensity as from Coumarin 152.

A practitioner of the photochemical arts will understand  
15 that the amount of each dye used to obtain this optical density may be extrapolated from commonly-available information on fluorescent and other dyes. For example, a table may show that the amount of Coumarin 152 necessary to realize an O.D. of 5.0  
20 at a particular wavelength is 6.5 mg./100 ml.; that the amount of rhodamine 110 required to realize the same O.D. is 2.6 mg./100 ml.; that the amount of sulforhodamine 640 required to realize the same O.D. is 2.2 mg./100 ml.; and that the amount of rhodamine 6G required to realize the same O.D. is 2.4 mg./100  
25 ml. Proportionate amounts are required to obtain other Optical Densities (O.D.).

Accordingly, the overall formulation by combined volume and weight measure -- whichever is easiest for the corresponding liquid or solid material -- is:

30	polymethylmethacrylate	70 ml.
	poly-2-hydroxymethyl methacrylate	30 ml.
	coumarin 152	.39 mg./100 ml.
	sulforhodamine 640	.152 mg./100 ml.
	rhodamine 110	.556 mg./100 ml.
35	rhodamine 6G	.144 mg./100 ml.

The polymer plastics are readily available from chemical supply houses. The preferred fluorophores, and many hundreds if not

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thousands of others (extending well beyond the common species listed in the Table 17) are available, inter alia, from Molecular Probes, Inc., telephone USA (503) 465-8353.

The Table 17 generally shows fluorophores, or fluorochromes -- all of which are known in the prior art -- that are suitable for use in the construction of fluorescent-type illuminator elements in accordance with the present invention.

#### 7.2 Preparation

Preparation of the illuminator elements is relatively straightforward. A solution of dyes is mixed with the polymerization initiator 2,2'-azobis(2-methyl-propionitrile). This prepared solution is added to, and mixed to homogeneity with, a polymer host stock solution. The preferred concentration of initiator was 3 mg/ml per final volume or reaction mixture.

The obtained composite material was phased into glass form, vacuumed and cured in a mold at a thermostatically-controlled temperature of 50°C during 24 hours.

The molded and formed polymer block with homogeneously dispersed mixture of fluorophores is removed from the form, typically cut to size, and then preferably polished to optical quality (at least on the surface that will face the specimen).

#### 7.3 A Matched Illuminator and Specimen Stain

An illuminator element having an illumination light output that is more intense at certain frequency or frequencies may serve to better resolve a specimen that is dyed with one or more dyes that transmit the frequency or frequencies (while absorbing other frequencies).

One example of such an illuminator having a light output that is tailored to a particular specimen stain may be constructed for use with the very common biological specimen stain eosin. Eosin is pink in color, having a  $\lambda_{\text{abs}}$  max = 525 nm. For contrast enhancement and "blue" and/or "red" emitting fluorophores can be used in making an associated illuminator. For example, the same "blue" and "red" component fluorophores may be used as are used in the making of the illuminator producing the preferred pseudo-white light. Namely, the blue is produced by coumarin 152 having a fluorescent absorbance maxima  $\lambda_{\text{abs}}$  (max)

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= 394 nm, and a fluorescent emission maxima  $\lambda_n(\text{max}) = 496$  nm. The "red" is produced by sulforhodamine 640 having a  $\lambda_{\text{abs}}(\text{max}) = 578$  nm and a  $\lambda_n(\text{max}) = 605$  nm. Both fluorophores are to a predetermined optical density in the mixture, and normally to the same optical density.

#### 7.4. Emission Spectra of the Preferred Embodiments of the Illuminators

An exemplary illuminator element of the present invention is constructed from as a polymers from a combination of (i) methyl methacrylate and (ii) 2-hydroxymethacrylate in approximate proportion 3:1 by volume, and more exactly in proportion 70 ml. to 30 ml. The polymer is excited with light that is substantially at a single frequency of, showing a very sharp peak of emission at, 366 nanometers. The polymer has some fluorescence in of itself, and shifts the 366 nanometers excitation light to substantially a single broad emission peaking at about 450 nanometers.

Another exemplary illuminator element may be made from (i) a liquid plastic consisting of two monomers, before polymerization, in combination with (ii) three fluorophores, as are used in construction of other particular illuminator elements in accordance with the present invention. Two preferred monomers are (i) methyl methacrylate and (ii) 2-hydroxymethacrylate in approximate proportion 3:1 by volume, and more exactly in proportion 70 ml. to 30 ml. The preferable three fluorophores, or fluorochromes, that are added are (i) coumarin 152 to an optical density of .3/cm, or approximately 6.5 mg. per 100 ml.; (ii) rhodamine 110 to an optical density of .3/cm, or approximately 2.6 mg. per 100 ml.; and (iii) rhodamine 610 to an optical density of .3/cm, or approximately 2.4 mg. per 100 ml. The mixture is again excited with light that is substantially at a single frequency of, showing a very sharp peak of emission at, 366 nanometers.

The emission spectrum of the monomers (i.e., the (i) methyl methacrylate and (ii) 2-hydroxymethacrylate) will change significantly upon their polymerization. The emission spectrum of each of the fluorophores will not significantly changed.

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Accordingly, the intensity of the emission spectra at lower nanometers wavelengths -- which intensity will be dominant before polymerization -- is not less than the peak at approximately 520 nm. resultant from rhodamine 110, nor another peak at 610 nm. resultant from rhodamine 610, in the final illuminator element. The emission peak at 496 nanometers from the coumarin 152 will not be so prominent as the emission peak so of the other two fluorophores. The relative strengths of all emission peaks may clearly be adjusted as desired or required in the production of colored light(s) in support of microscopy.

Yet another illuminator element having an exact equivalent spectrum of emission of the illuminator element just discussed -- in other words the polymerized body with the three fluorophores -- may be made by the addition that titanium dioxide ( $\text{TiO}_2$ ), typically in an amount of 10 mg. per 100 ml. A darkfield illuminator will thus be made. In such an illuminator, and regardless of where upon the surface of the darkfield illuminator fluorescent light is emitted and howsoever such light varies in intensity over the surface, the relative intensity at varying frequencies (as well as the relative intensity at various spatial positions relative to the illuminator) will be much affected. The lower wavelength fluorescent emission of the coumarin 152 is either (i) but relatively poorly reflected by the  $\text{TiO}_2$  and/or (ii) but poorly self-absorbed. In fact, it is condition (ii) that prevails. Meanwhile, both varieties of the rhodamine are good self-absorbers of their own fluorescent emissions.

Moreover, the inclusion of light-scattering bodies (i.e., the titanium dioxide) -- especially in densities that promote the realization of a darkfield illuminator element -- also serves to change the frequency mixture of the emitted light. One fluorophore may be better, or worse, at self-absorbing its own emission(s), or the emission(s) of another fluorophore. The entire mixture becomes a "witches brew", with considerable planning and skill being useful in the selection, and the blending, of plural fluorophores and also reflecting bodies to obtain illumination light output of the desired spectral

characteristics.

#### 7.5 Brightfield and Darkfield Illuminator Elements

A diagrammatic representation of a brightfield illuminator element in accordance with the present invention is shown in Figure 16a. The arrows at shown at only a single, top, surface of the brightfield illuminator element are intended not to represent the directions of the light emissions, which emissions are directionally isotropic and random (compare, for example, Figures 9 and 11), but only that the brightfield illuminator element produces a relatively even magnitude of illumination at its top surface. (Accordingly, the meaning of these arrows is different than those ray trace arrows shown to proceed from the external light source, or light bulb, to the illuminator element in the same Figure 16a.) It is this top surface that is, arbitrarily in Figure 16, the surface that is positionally disposed towards the specimen under observation.

Conversely, a diagrammatic representation of a darkfield illuminator element in accordance with the present invention is shown in Figure 16b. The darkfield illuminator element produces a relatively more intense illumination at the peripheral, as opposed to the central, regions of its top surface. This is again intended to be illustrated by the varying length of the arrows exiting the top surface.

#### 8. Comparison to Koehler Illumination

As stated in section 5 of the SUMMARY OF THE INVENTION portion of this specification, the specimen illumination obtainable with the illuminator elements of the present invention directly fulfills many of the requirements, and is analogous to satisfying certain remaining requirements, of Koehler illumination. Yet it is not precise to say that the illumination provided by the illuminator element of the present invention is precisely Koehler illumination because Koehler illumination deals with the requirements of the different, traditional, illumination of a specimen by use of condensers.

From section 2.2 of the BACKGROUND OF THE INVENTION portion of this specification, it will be recalled that a first criteria of Koehler illumination is that the field should be homogeneously

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bright. This criteria is completely met by the illuminator elements of the present invention.

5 The second criteria of Koehler illumination is that the working numerical aperture of the condenser and the size of the illuminated field can be regulated independently. There is, of course, no condenser used, required or desired with the illuminator elements of the present invention, which completely replace condensers. The size of the illuminated field can, however, be regulated independently of anything else.  
10 Accordingly, this criteria of Koehler illumination is deemed to be partially met by the illuminator elements of the present invention because the numerical aperture of an illuminator element is fixed.

15 The third criteria of Koehler illumination is that the specimen should be illuminated by a converging set of plane wave fronts, each arising from separate points. This was previously obtained by imaging of the light source in the condenser aperture. Now, with the illuminator elements of the present invention, the planar wave fronts of light arise from sources,  
20 such as chromophores or light-scattering bodies, that are spatially distributed. Accordingly, this criteria of Koehler illumination is manifestly met by the illuminator elements of the present invention.

25 The fourth and fifth criteria of Koehler imaging are really expressions of results. Koehler imaging gives rise to the maximum lateral resolution and very fine optical sectioning, which yields maximum axial resolution. Meanwhile, the front focal plane of the condenser becomes conjugate with the rear focal plane of the objective lens, a condition needed for optimal  
30 contrast enhancement of the finer specimen details. These results are non-analogous to the illumination, and the illuminating devices, of the present invention. Accordingly, Koehler illumination cannot be directly compared in this regard.

35 The sixth and final, criteria of Koehler illumination is that any flare arising from the microscope optics and their barrels is reduced without any vignetting. The illuminator



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elements of the present invention fully accomplish this also.

9. Future Modifications

In accordance with the preceding explanation, variations and adaptations of the illuminator in accordance with the present invention will suggest themselves to a practitioner of the optical and optical materials arts.

For example, chemicals and/or other material that does not interfere with the function of the illuminator elements may be added to the matrix. Such chemicals and materials may serve, when appropriately analyzed, as an indication of origin, and authenticity, of a particular illuminator element.

In order to improve performance, cost effectiveness and/or service to particular applications, several modifications of the basic illuminator elements in accordance with the present invention are possible. Some of these are:

The geometry of the illuminator may be optimized for either and/or for both of (i) the reception of primary radiation from an external light source, or (ii) the coupling of illumination onto a particular specimen. For example, a horn-shaped cavity in the illuminator element, the opening of which cavity was directionally disposed towards the external light source, would cause, by multiple reflections, that almost all of the light from the external light source would enter into the illuminator.

Selective surfaces of the illuminator element may be mirrored.

The choice of fluorophores, or of combinations of fluorophores, within an illuminator element may be optimized for illumination according to characteristics of specimen (staining, own fluorescence, thickness, etc.).

A special holder is possible for using an illuminator element with upright microscope. A low power mercury lamp may be used for epi-illumination with "non-epi" microscopes.

Finally, many variations are possible on the darkfield illuminator element, and in the range between the brightfield and the darkfield illuminator elements. The central core of an illuminator element, for example an elongate "plug" to a cylindrically-shaped illuminator element, could be filled with

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various fluorophores and/or light-absorbing dyes. Meanwhile, the surrounding volume could be filled with fluorophores of a contrasting color and/or light-scattering bodies. Although the specimen would still be bathed in directionally isotropic light, such light would not be of the identical color from all directions. Specimen features absorbing one color light might throw a shadow in one direction; specimen features absorbing a complimentary color light might throw a shadow in the other direction. The height, or size, of the plural, and plural-colored, specimen features might thus usefully be compared in a manner not obtainable with simple brightfield illumination.

In accordance with these and other possible variations and adaptations of the present invention, the scope of the invention should be determined in accordance with the following claims, only, and not solely in accordance with those particular embodiments within which the invention has been taught.

TABLE OF KNOWN FLUOROCHROMES/FLUOROPHORES  
SUITABLE FOR USE IN CONSTRUCTION OF ONE EMBODIMENT OF  
AN ILLUMINATOR ELEMENT OF THE PRESENT INVENTION

<u>FLUOROCHROME</u>	<u>EXCITATION</u>	<u>EMISSION</u>
3-Hydroxypyrene 5,8,10-Tri Sulfonic acid	403	513
5-Hydroxy Tryptamine	380-415	520-530
5-Hydroxy Tryptamine (5-HT)	400	530
Acid Fuchsin	540	630
Acridine Orange (bound to DNA)	502	526
Acridine Red	455-600	560-680
Acridine Yellow	470	550
Acriflavin	436	520
AFA (Acriflavin Feulgen SITSA)	355-425	460
Alizarin Complexon	530-560	580
Alizarin Red	530-560	580
Allophycocyanin	650	661
ACMA	430	474
Aminoactinomycin D	555	655
Aminocoumarin	350	445
Anthroyl Stearate	361-381	446
Astrazon Brilliant Red 4G	500	585
Astrazon Orange R	470	540
Astrazon Red 6B	520	595
Astrazon Yellow 7 GLL	450	480
Atabrine	436	490
Auramine	450-490	550
Aurophosphine	450-490	515
Aurophosphine G	450	580
BAO 9 (Bisaminophenyloxadiazole)	365	395
BCECF	505	530
Berberine Sulphate	430	550
Bisbenzamide	360	600-610
BOBO 1	462	481
Blancophor FFG Solution	390	470
Blancohor SV	370	435
Bodipy Fl	503	512
BOPRO 1	462	481
Brilliant Sulphoflavin FF	430	520
Calcien Blue	370	435
Calcium Green	505	532
Calcofluor RW Solution	370	440
Calcofluor White	440	500-520
Calcophor White ABT Solution	380	475
Calcophor White Standard Solution	365	435
Cascade Blue	400	425
Catecholamine	410	470
Chinacrine	450-490	515
Coriphosphine O	460	575
Coumarin-Phalloidin	387	470
CY3.18	554	568
CY5.18	649	666
CY7	710	805
1-Dimethyl Amino Naphaline 5 Sulphonic Acid	340	525

Table 17a

TABLE OF KNOWN FLUOROCHROMES/FLUOROPHORES  
SUITABLE FOR USE IN CONSTRUCTION OF ONE EMBODIMENT OF  
AN ILLUMINATOR ELEMENT OF THE PRESENT INVENTION

<u>FLUOROCHROME</u>	<u>EXCITATION</u>	<u>EMISSION</u>
Dansa (Diamino Naphtyl Sulphonic Acid)	340-380	430
Dansyl NH-Ch3 in water	340	578
DAPI	350	470
Diamino Phenyl Oxydiazle (DAO)	280	460
Dimethylamino-5-Sulphonic acid	310-370	520
Diphenyl Brilliant Flavine 7GFF	430	520
Dopamine	340	490-520
Eosin	525	545
Erythrosin ITC	530	558
Ethidium Bromide	510	595
Euchrysin	430	540
FIF (Formaldehyde Induced Fluorescence)	405	435
Flazo Orange	375-530	612
Fluorescein Isothiocyanate (FITC)	490	525
Fluo 3	485	503
Fura-2	340-380	512
Genacryl Brilliant Red B	520	590
Genacryl Brilliant Yellow 10GF	430	485
Genacryl Pink 3G	470	583
Genacryl Yellow 5GF	430	475
Gloxalic Acid	405	460
Granular Blue	355	425
Haematophorphyrin	530-560	580
Hoechst 33258 (bound to DNA)	346	460
Indo-1	350	405-482
Intrawhite Cf Liquid	360	430
Leucophor PAF	370	430
Leucophor SF	380	465
Leucophor WS	395	465
Lissamine Rhodamine B200 (RD200)	575	595
Lucifer Yellow CH	425	528
Lucifer Yellow VS	430	535
Magdala Red	524	600
Maxilon Brilliant Flavin 10 GFF	450	495
Maxilon Brilliant Flavin 8 GFF	460	495
MPS (Methyl Green Pyronine Stilbene)	364	395
Mithramycin	450	570
NBD Amine	450	530
Nile Red	515-530	525-605
Nitrobenzoxadidole	460-470	510-650
Noradrenaline	340	490-520
Nuclear Fast Red	289-530	580
Nuclear Yellow	365	495
Nylosan Brilliant Flavin E8G	460	510
Pararosaniline (Feulgen)	570	625
Phorwite AR Solution	360	430
Phorwite BKL	370	430
Phorwite Rev	380	430
Phorwite RPA	375	430
Phospine 3R	465	565

Table 17b

TABLE OF KNOWN FLUOROCHROMES/FLUOROPHORES  
SUITABLE FOR USE IN CONSTRUCTION OF ONE EMBODIMENT OF  
AN ILLUMINATOR ELEMENT OF THE PRESENT INVENTION

<u>FLUOROCHROME</u>	<u>EXCITATION</u>	<u>EMISSION</u>
Phycoerythrin R	480-565	578
Pontochrome Blue Black	535-553	605
Primuline	410	550
Procion Yellow	470	600
Propidium Iodide	536	617
Pyronine	410	540
Pyronine B	540-590	560-650
Pyrozal Brilliant Flavin 7GF	365	495
Quinacrine Mustard	423	503
Rhodamine 123	511	534
Rhodamine 5 GLD	470	565
Rhodamine 6G	526	555
Rhodamine B	540	625
Rhodamine B 200	523-557	595
Rhodamine B Extra	550	605
Rhodamine BB	540	580
Rhodamine BG	540	572
Rhodamine WT	530	555
Rose Bengal	540	550-600
Serotonin	365	520-540
Sevron Brilliant Red 2B	520	595
Sevron Brilliant Red 4G	500	583
Sevron Brilliant Red B	530	590
Sevron Orange	400	530
Sevron Yellow L	430	490
SITS (Primuline)	395-425	450
SITS (Stilbene Isothiosulphonic acid)	365	460
Stilbene	335	440
Snarf 1	563	639
Sulpho Rhodamine B Can C	520	595
Sulpho Rhodamine G Extra	470	570
Tetracycline	390	560
TRITC (Tetramethyl Rhodamine Isothiocyanate)	557	576
Texas Red	596	615
Thiazine Red R	510	580
Thioflavin S	430	550
Thioflavin TCN	350	460
Thioflavin 5	430	550
Thiolyte	370-385	477-484
Thiozol Orange	453	480
Tinopol CBS	390	430
TOTO 1	514	533
TOTO 3	642	661
True Blue	365	420-430
Ultralite	656	678
Uranine B	420	520
Uvitex SFC	365	435
Xylene Orange	546	580
XRITC	582	601
YO PRO 1	491	509

Table 17c

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## CLAIMS

What is claimed is:

1. An illuminator element for use with an optical microscope comprising:

5 a source of spatially isotropic light suitably sized and shaped so as to be positioned closely proximate to a specimen under observation by a microscope, therein to illuminate the specimen so substantially spatially isotopically that no shadows are visible through the microscope.

10 2. The illuminator element according to claim 1 wherein the spatially isotropic illuminating light is substantially only at single predetermined narrow range of frequencies as constitutes a single color.

15 3. The illuminator element according to claim 1 wherein the spatially isotropic illuminating light is substantially only at a plurality of predetermined narrow ranges of frequencies each of which ranges constitutes a single color.

20 4. The illuminator element according to claim 1 wherein the spatially isotropic illuminating light is substantially only at the plurality of predetermined narrow ranges of frequencies is a pseudo-white light.

5. The illuminator element according to claim 1 wherein the source of spatially isotropic light comprises:

25 a great multiplicity of substantially-non-directional substantially-molecular-size light sources; evenly spatially distributed within

a stable matrix suitably sized and shaped so as to be positioned closely proximate to the specimen under observation by the microscope;

30 wherein the even distribution of the light sources coupled with their substantial lack of any preferred direction produces the substantially spatially isotopic illumination of the

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proximate specimen.

6. The illuminator element according to claim 5 wherein at least one of the great multiplicity of light sources comprises:  
a luminescent light source emitting light.

5 7. The illuminator element according to claim 5 wherein the at least one luminescent light source derives an energy of its light emission from within the stable matrix.

8. The illuminator element according to claim 7 wherein the at least one luminescent light source comprises:  
10 a radioluminescent light source deriving its energy of light emission from a decay of radionuclides that are within the stable matrix.

9. The illuminator element according to claim 7 wherein the at least one luminescent light source comprises:  
15 a chemiluminescent light source deriving the energy of light emission from a chemical reaction that transpires within the stable matrix.

10. The illuminator element according to claim 5 wherein at least one luminescent light source derives an energy of its light  
20 emission from energy that is externally supplied to the stable matrix.

11. The illuminator element according to claim 5 wherein at least one luminescent light source deriving the energy of its light emission from energy that is externally supplied to the  
25 stable matrix so derives energy from light radiation that is externally supplied to the stable matrix.

12. The illuminator element according to claim 11 wherein the at least one luminescent light source comprises:  
a fluorescent light source.

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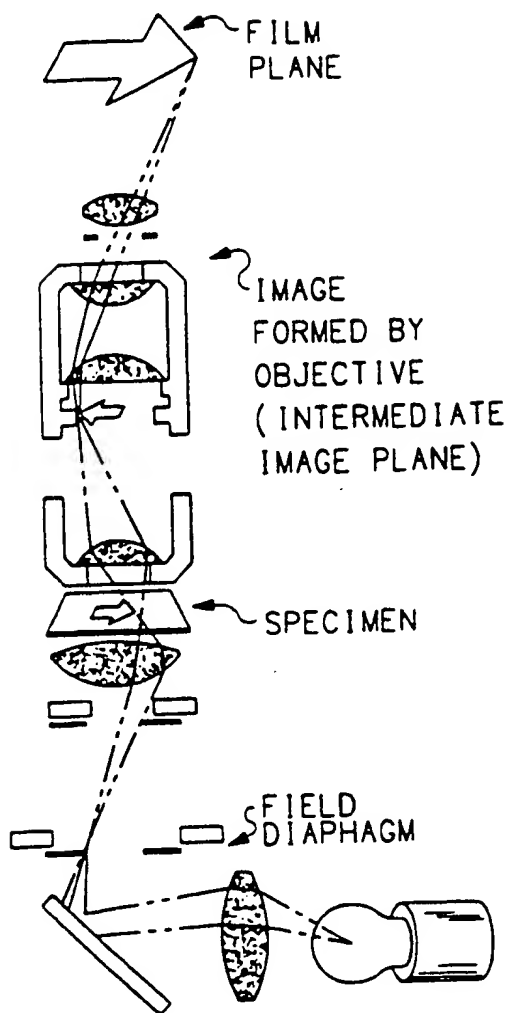
13. The illuminator element according to claim 5 wherein at least some of the great multiplicity of light sources comprise:

a multiplicity of light-scattering bodies randomly evenly distributed within the spatial matrix;

5        wherein a non-isotropic light externally supplied to the illuminator element is randomly scattered multiple times between random ones of the multiplicity of light-scattering bodies within the stable matrix so as to exit the stable matrix as the substantially spatially isotropic light regardless that the  
10        externally supplied light is not itself isotropic.



FIG. 2  
PRIOR ART



1/8

FIG. 1  
PRIOR ART

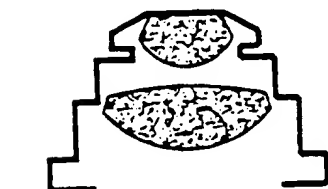
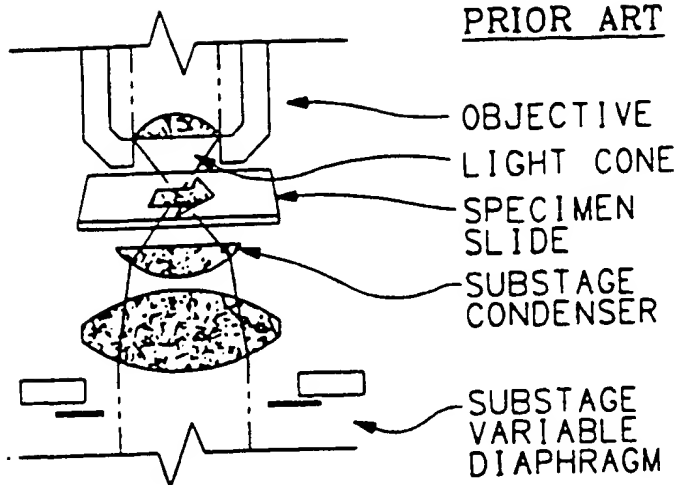


FIG. 3a  
PRIOR ART

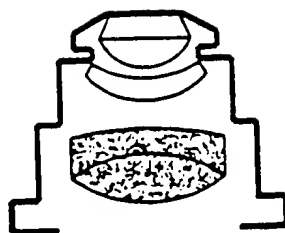


FIG. 3b  
PRIOR ART

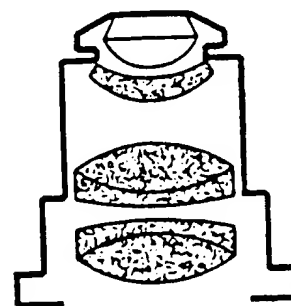


FIG. 3c  
PRIOR ART

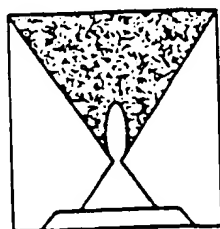


FIG. 4a  
PRIOR ART

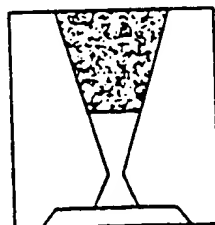


FIG. 4b  
PRIOR ART

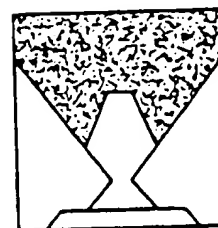


FIG. 4c  
PRIOR ART

FIG. 5  
PRIOR ART

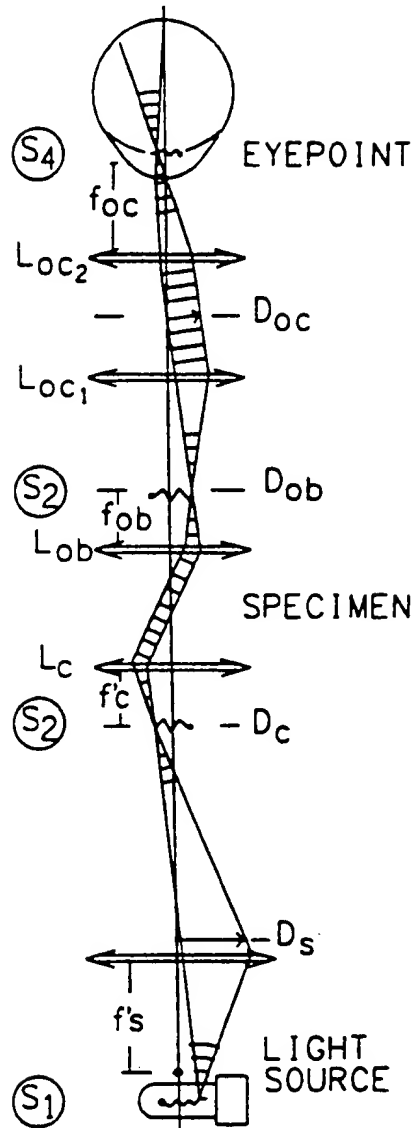
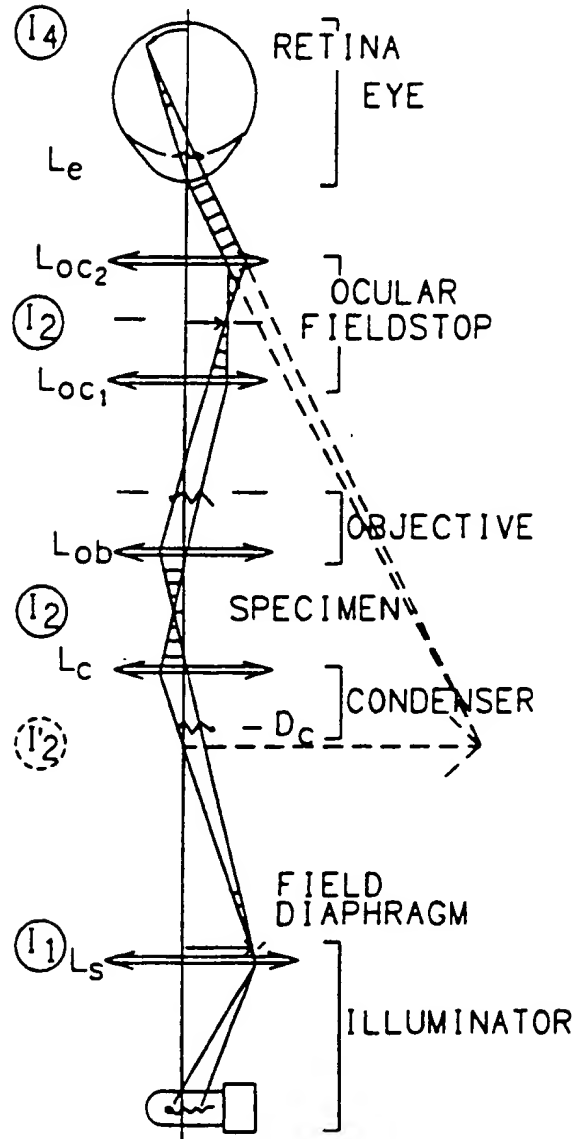


FIG. 6  
PRIOR ART



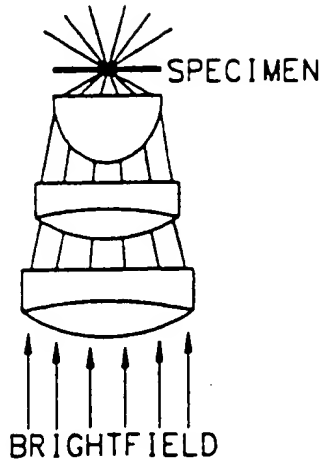


FIG. 7a  
PRIOR ART

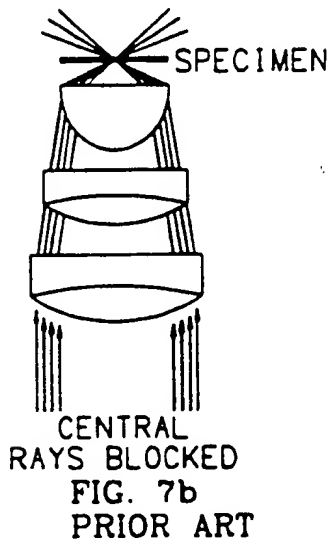


FIG. 7b  
PRIOR ART

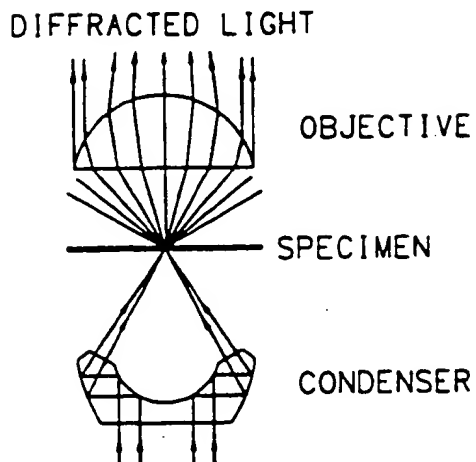


FIG. 7c  
PRIOR ART

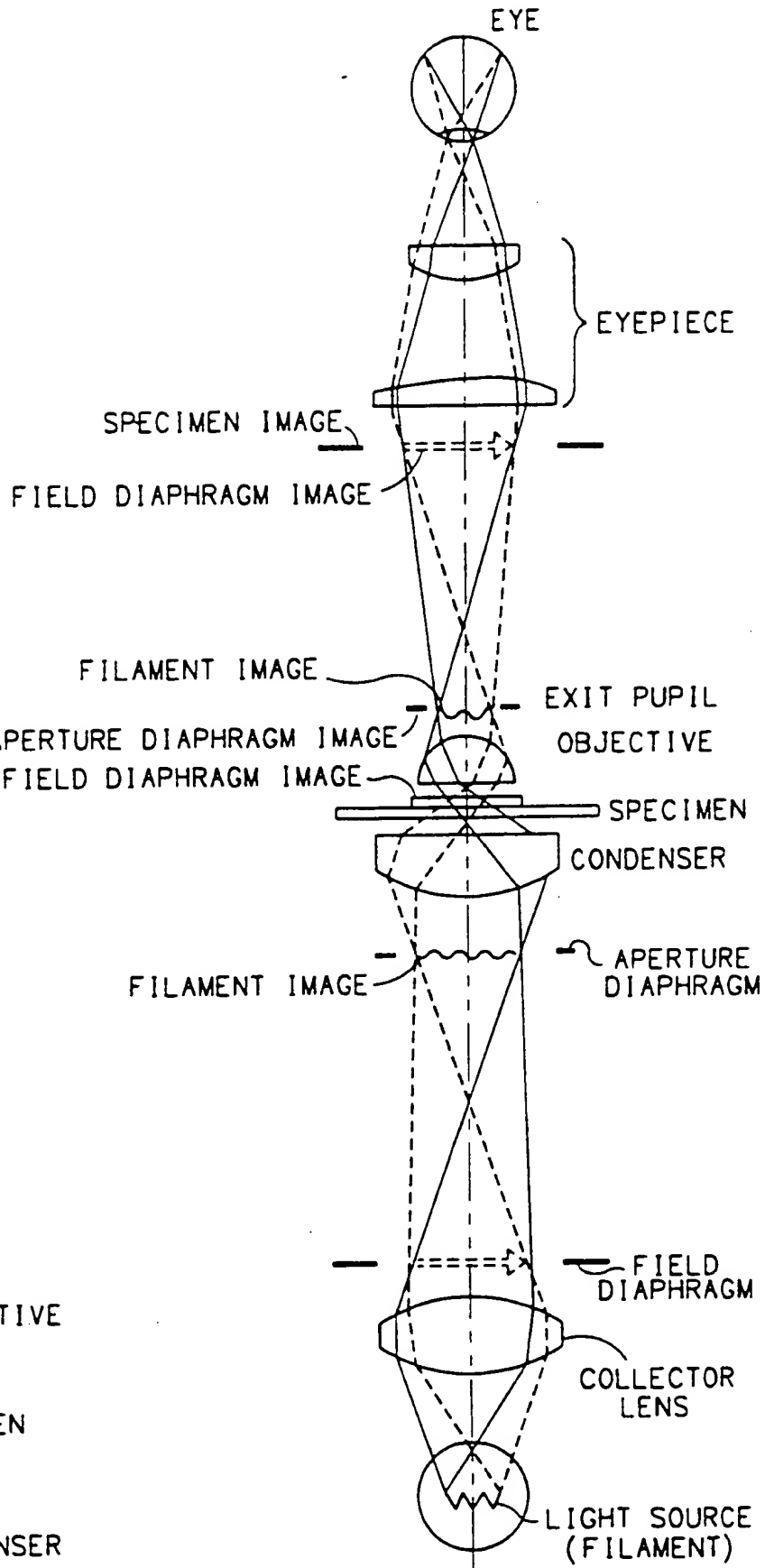


FIG. 8  
PRIOR ART

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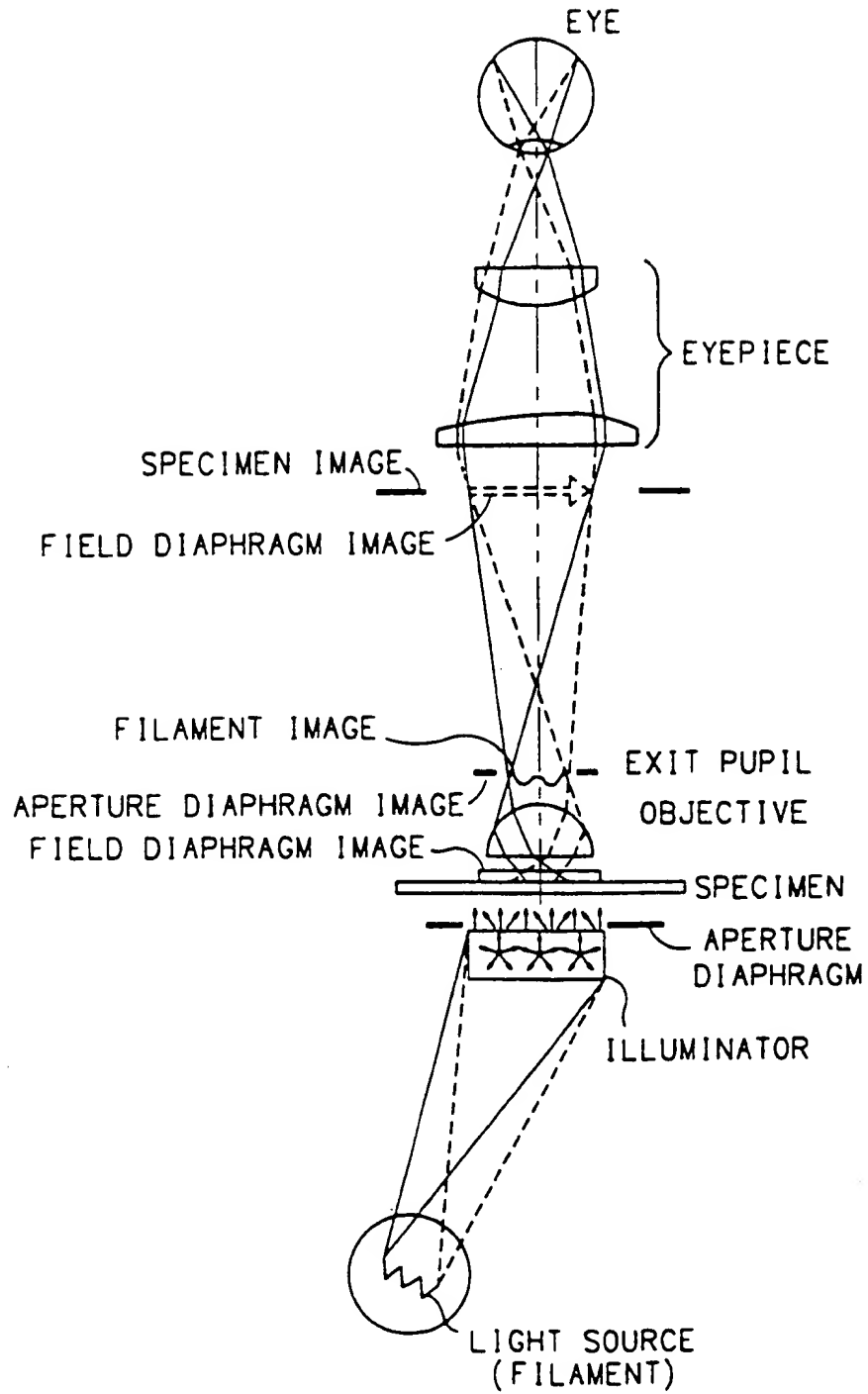


FIG. 9

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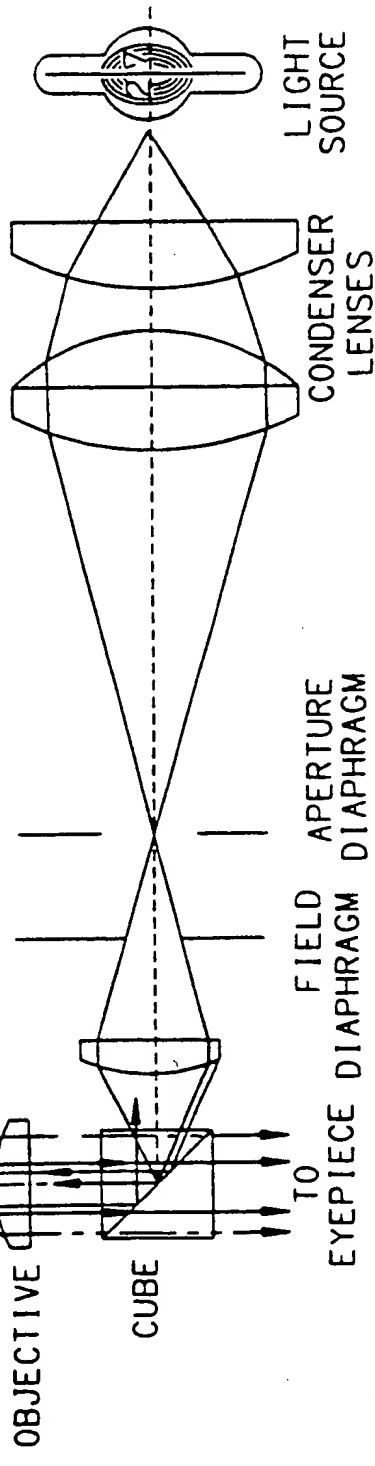
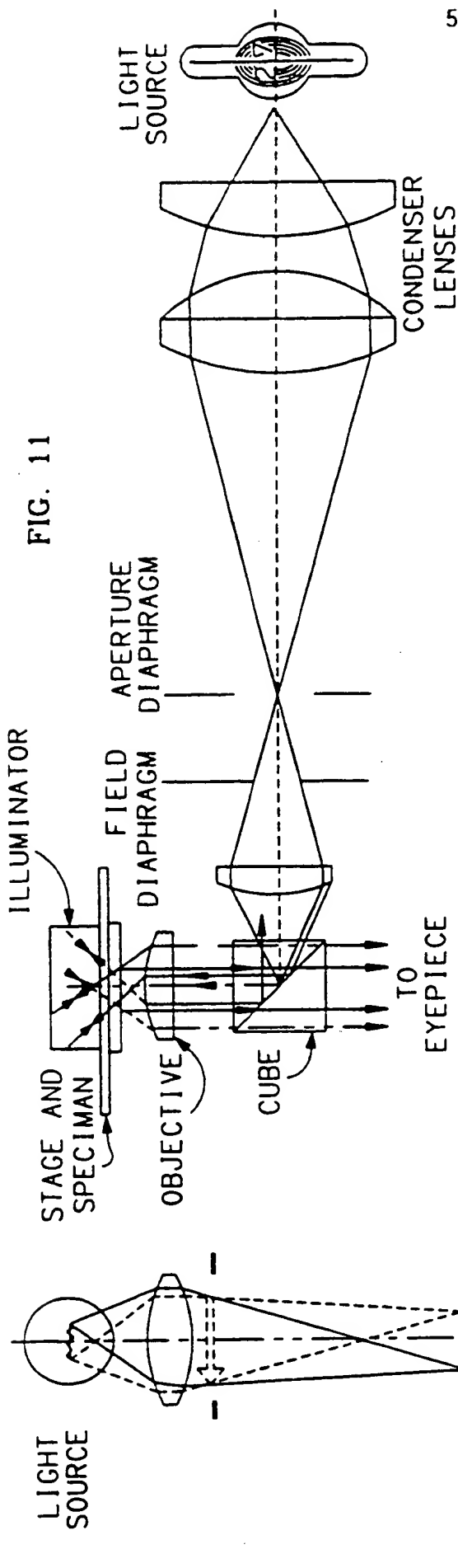


FIG. 10  
PRIOR ART

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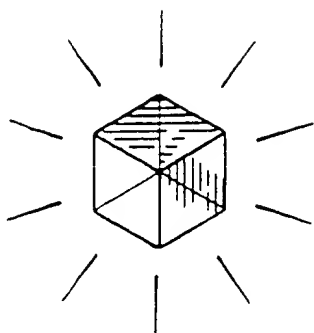


FIG. 12a

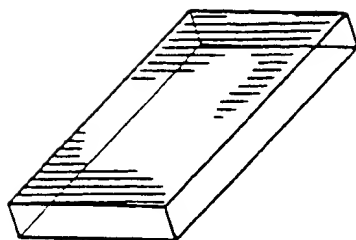


FIG. 12b



FIG. 12c

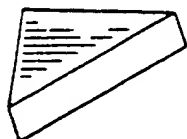


FIG. 12d

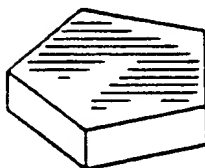


FIG. 12e

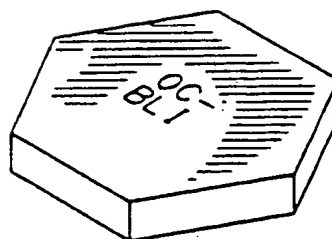


FIG. 12f

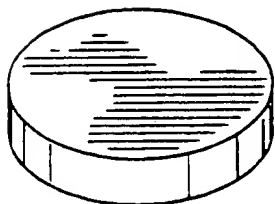


FIG. 12g

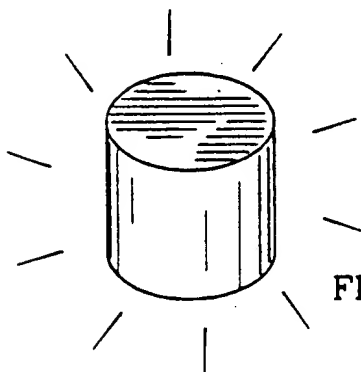


FIG. 12h

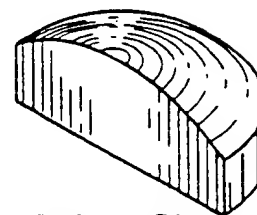


FIG. 12i

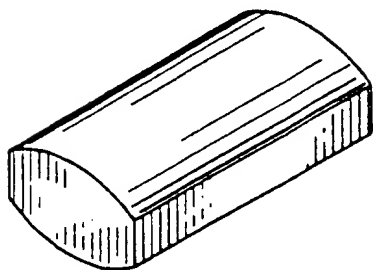


FIG. 12j

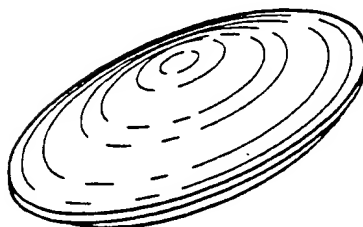


FIG. 12k

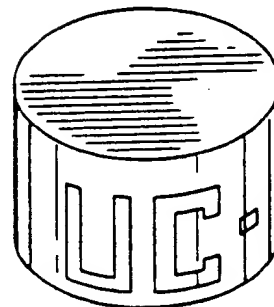


FIG. 12l

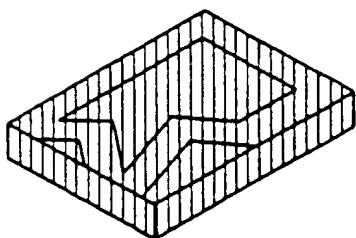


FIG. 12m

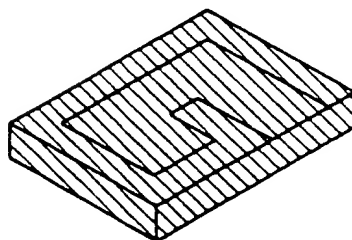


FIG. 12n

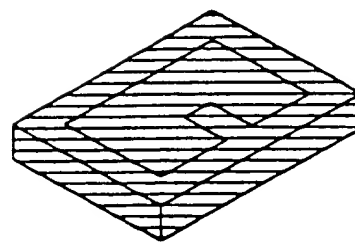
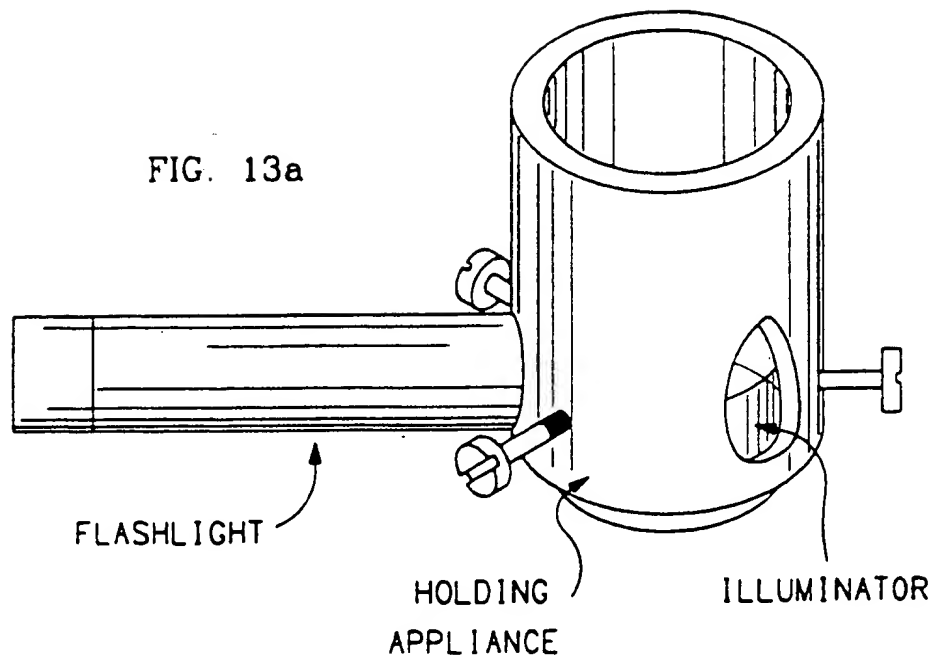


FIG. 12o

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FIG. 13a



HOLDING  
APPLIANCE

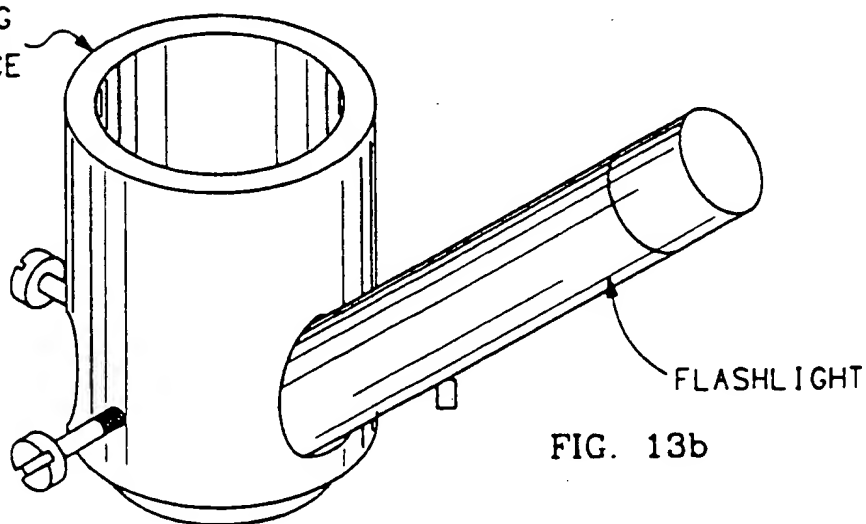


FIG. 13b

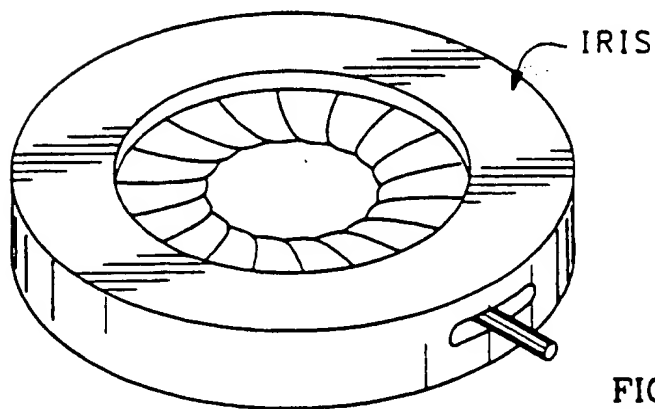


FIG. 14  
PRIOR ART

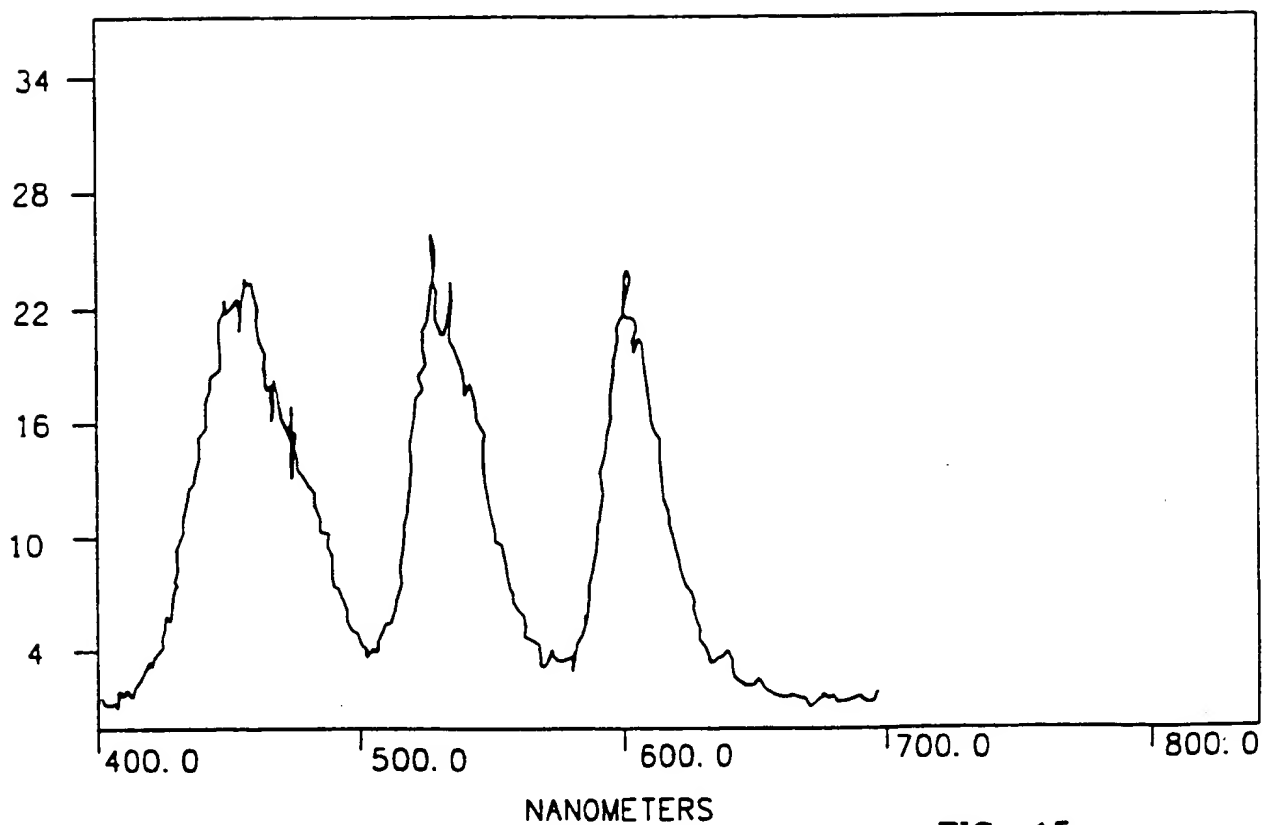
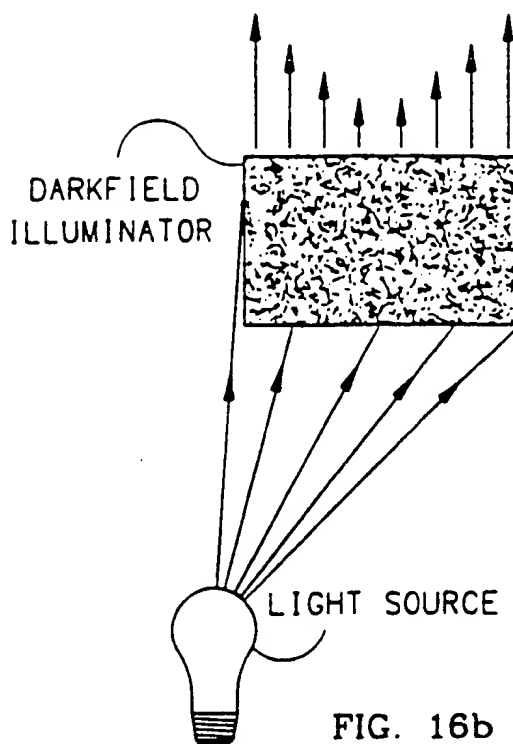
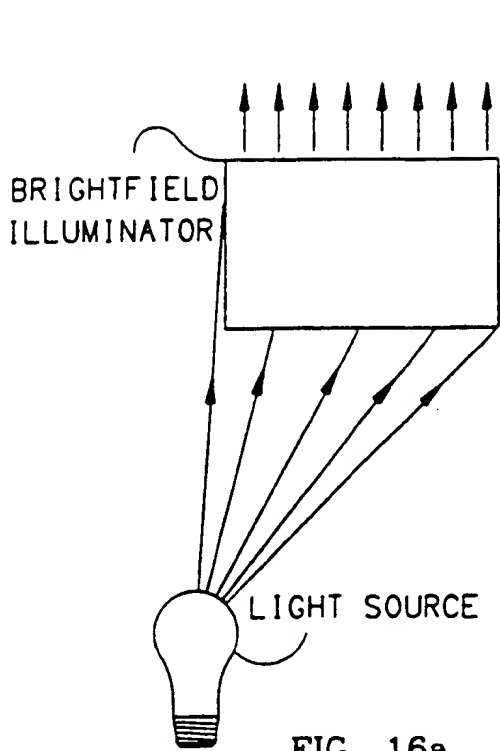


FIG. 15



## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US95/06085

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : G02B 21/00, 21/06.

US CL : 359/368, 385-390, 618.

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 359/368, 385-390, 618.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched  
NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

ILLUMINATI? OR ILLUMINATOR?, MICROSCOPE, ISOTROP?, LUMINESCEN? OR FLUORESCEN?,  
MOLECULAR?(5A)LIGHT, MATRIX, NARROW(5A)FREQUENC?, SHADOW?

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X/Y	US, A 4,662,747 (ISAACSON ET AL) 5 May 1987, columns 8-9, and 11 and figs. 3-6.	(1-3)/(4-13)
X/Y	US, A 4,947,034 (WICKRAMASINGHE ET AL) 7 AUGUST 1990, columns 2-4 and figs. 3-4.	(1-2)/(3-4)
Y	US, A 4,948,247 (LAPEYRE) 14 AUGUST 1990, columns 4 and 6 and fig. 1.	1-2
A	US, A 4,665,036 (DEDDEN ET AL) 12 MAY 1987, the whole documentation.	1-13
A	US, A 5,260,826 (WU) 9 NOVEMBER 1993, the whole documentation.	1-13

☐ Further documents are listed in the continuation of Box C.
 ☐ See patent family annex.

* Special categories of cited documents:	*T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*E* earlier document published on or after the international filing date	*Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*G*	document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means		
*P* document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

20 JULY 1995

Date of mailing of the international search report

31 JUL 1995

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